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INFLUENCE OF PROCESSING DESIGN ON THE ACCESSIBILITY OF PHENOLICS IN BLUEBERRY (*VACCINIUM CORYMBOSUM* L.) PURÈES: THE EFFECT OF HYDRATION AND MICROSTRUCTURAL PROPERTIES

Scientific field AGR/15

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ABSTRACT

Fresh blueberry (*Vaccinium corymbosum* L.) fruits are one main source of phenolic phytochemicals and derived frozen purées could be considered valuable and versatile products, being processed without pomace loss and potentially rich in all the bioactive compounds which are characteristic of the whole berry. Colour, flavour and health-promoting attributes make these products precious ingredients in food preparations and in particular in the increasing market of fruit blended beverages.

Fruit and vegetable liquid-like products are perceived by consumers as healthy and convenience foods, indeed a lack of knowledge still exists on phenomena which link process to functions in these matrices. Hence scientific research on plant-derived food suspensions has been growing in these last few years, mainly addressed to the study of functional and nutritional properties of vegetable-based puréed products, such as sauces, pastes and soups. In these matrices, under the effect of thermal and mechanical forces, native plant tissues lose continuity, a new system of solid particles in viscous liquids is shaped and bioactive molecules find a new arrangement all through the liquid and particle phase of the newly-formed system. Cell wall materials interact both with water and solutes in plant-food matrices, thus affecting the fate of dispersed bioactive components consumed through diet. Hence distribution of phenolic constituents between particle and liquid components of plant food represent a relevant technological and nutritional variable. Nevertheless few studies are available which describe the influence of processing on these parameters in real food systems and in particular in fruit matrices

In the case of blueberry-based suspensions, despite the well assessed health-promoting attributes of the fruits and the popularity of smoothie products, very few studies are available which combine the analysis of bioactive constituents to the study of their spatial arrangement throughout the liquid-food system and explore the process–structure–function relations.

The purpose of this thesis was to investigate the influence of mechanical and thermal treatments on phenolic-delivering properties of blueberry-derived purées, taking into account both the total content in phenolic bioactive compounds and the proportion of particle bound to dissolved phenolic constituents. To this aim a complementary physical/chemical, microstructural and biochemical approach was applied.

The first step of this research (Topic 1) was to draw up a processing plan for producing blueberry-derived homogenates by combining mechanical and thermal treatments of increasing intensities with heat treatments planned either before or after the homogenization steps. By acting on blanching(BL)/homogenisation (H) times and operation order (BL+H; H+BL) and replicating the processing in three consecutive days, three sets of 23 different fruit/homogenate samples were obtained from individually quick frozen blueberries.

The second part of this research (Topic 2) examined the effects of the order and duration of processing operations on chemical/physical and hydration properties of the products. The strength by which homogenate matrices retain their liquid fraction limits both mobility of bioactive metabolites dissolved within matrix and syneresis phenomena, hence affecting sensorial, textural as far as nutritional attributes of the product. The amount of serum released from homogenate samples under gravity (G), under the combined effect of gravity and weak capillary suction force (FP) and under centrifugal force (C) were measured and the volume at maximum hydration (SC, swelling capacity) of particle components was registered. A measure of water activity (a_w) was provided and main quality parameters were assessed. Results indicate that liquid retaining feature of purées increased with BL time, and that BL+H purées had higher amounts of entrapped or loosely bound water than H+BL ones, indicating that operation order

might act on tightly-bound water, whereas the blanching time on the loosely-bound water. The effect of mechanical forces on dynamic volume of CWM was much more pronounced in BL+H samples because fruit fibers had been preliminary loosened by heat treatments, whereas in HBL purées the preliminary H-treatments increased the surface area of CWM but not their dynamic volume (SC).

The third part of the research (Topic 3) was dedicated to assess the influence of processing on the composition in anthocyanin and phenolic molecules of the liquid (easily accessible) and particle (scarcely accessible) fractions of the homogenate systems. Dissolved phenolic compounds were collected within the liquid fraction separated under natural or enhanced gravity from the products, while polyphenols associated to the tissue particles were extracted by selected solvent. Liquid fractions and extracts were analyzed for total phenolic compounds, colour indices as far as for total and individual anthocyanin compounds by applying spectrophotometric and chromatographic techniques. Results underlined that in the intact fruit system TPC and MAP did not change with BL, which, on the other hand, could enhance the release of TPC from fruit native compartments and/or protect them from oxidative degradation during the H step. In contrast, the losses of TPC and MAP associated to the initial H step could not be compensated by the successive BL step. In purées higher particle-bound to total TPC and MAP ratios were found for H+BL samples. DAD-HPLC analysis indicated that the higher recovery of anthocyanin compounds in BLH compared to HBL purées was related to the protective effect exerted by preliminary berry blanching towards delphynidins and petunidins, most reactive and unstable anthocyanin compounds. Furthermore, in the particle-liquid homogenate systems, delphynidins were mainly associated to cell wall materials and malvidins to the serum phase.

Most of the investigated chemical/physical and biochemical phenomena are played at the microscale level of blueberry fruit cell walls and membranes. Thus the last part of the research (Topic 4) was dedicated to the study of microstructure of the samples. Homogenate particle components were separated by sedimentation trials and histochemical features of raw and thermally treated blueberry fruits and homogenate particles were analyzed by fluorescence microscopy tools. In BL+H products single cells were obtained, the continuity of membranes and walls was better preserved, and the ultrastructure of cell walls deeply altered with cellulose fibrils rippening off wall surface produced a widespread network of fibrillary cellulose increasing the active surface area of the matrix. In H+BL products larger and entangled cluster of cells were obtained, the continuity of both cell wall and membranes was lost, cytoplasmic material leaked out from the broken edges, producing a lower phase volume of the particle phase as well as higher amounts of free cytoplasmic materials.

RIASSUNTO

I frutti di mirtillo (*Vaccinium corymbosum* L.) costituiscono una importante fonte naturale di composti fenolici e puree congelate preparate da queste bacche possono essere considerate prodotti versatili a elevato valore aggiunto, ottenuti senza che ci siano perdite dovute a scarti di lavorazione e, quindi, potenzialmente ricchi di tutti i composti bioattivi che sono caratteristici della bacca. Colore, flavour e proprietà salutistiche rendono le puree di mirtillo congelate prodotti utili nel settore in crescita della "frutta da bere" (smoothies).

I prodotti liquidi a base di frutta e ortaggi sono percepiti dai consumatori come salutari e "comodi", poiché già pronti per il consumo. Tuttavia i fenomeni che legano i processi di lavorazione alle proprietà funzionali in questo tipo di matrici non sono ancora stati approfonditi. Per questo motivo negli ultimi anni è cresciuta la ricerca nel settore delle sospensioni alimentari, in particolare volta allo studio delle proprietà nutrizionali e funzionali di puree a base vegetale come salse, puree e zuppe. Per effetto dei trattamenti meccanici e termici, in queste matrici i tessuti vegetali originari perdono la loro continuità, si origina un nuovo sistema costituito da particelle solide in liquidi viscosi e le molecole bioattive trovano una nuova ripartizione tra la fase liquida e il particolato. I polimeri delle pareti cellulari (CWM), interagendo sia con l'acqua che con i soluti, influenzano il destino alimentare dei componenti bioattivi dispersi nella nuova matrice.

La ripartizione dei composti fenolici tra le componenti solida e liquida del prodotto vegetale costituisce quindi una variabile tecnologica e nutrizionale rilevante.

Nonostante l'importanza di questi aspetti, esistono pochi studi che esplorino le relazioni tra processo di trasformazione, microstruttura e funzionalità in matrici reali e, in particolare, nei prodotti da frutta derivati da mirtillo..

Nel presente progetto è stata studiata l'influenza dei trattamenti termici e meccanici sulla disponibilità e accessibilità dei composti fenolici in puree di mirtillo, considerando sia il contenuto totale dei composti fenolici bioattivi sia la proporzione tra costituenti fenolici legati alle particelle e dissolti, approfondendo, con un approccio complementare, gli aspetti biochimici, fisico-chimici e microstrutturali.

Nel primo stadio di questa ricerca (Topic 1) si è redatto uno schema del processo di trasformazione per la produzione di omogenati di mirtillo combinando trattamenti meccanici e termici di intensità crescente e pianificando i trattamenti termici prima o dopo le fasi di omogenizzazione. Agendo sui tempi di scottatura (BL) e di frullatura (H) e sull'ordine di queste fasi operative e replicando il piano di trasformazione in tre giorni consecutivi, si sono ottenute, da mirtilli previamente sottoposti a surgelazione rapida individuale, tre serie di 23 campioni di frutti/omogenati differenti.

Nella seconda parte di questa ricerca (Topic 2) sono stati esaminati gli effetti dell'ordine e della durata delle operazioni di scottatura e omogenizzazione sulle proprietà chimico-fisiche e di idratazione dei prodotti. La forza con cui la matrice dell'omogenato trattiene la frazione liquida limita la mobilità dei metaboliti bioattivi dissolti nella matrice e i fenomeni di sineresi, influenzando, quindi, le caratteristiche sensoriali, strutturali e nutrizionali del prodotto. È stata quindi misurata la quantità di siero rilasciata dai campioni omogenati per gravità (G), per effetto combinato di gravità e capillarità (FP) e per centrifugazione (C), come pure il volume alla massima idratazione delle particelle (SC, capacità di rigonfiamento), l'attività dell'acqua (a_w) e i principali parametri qualitativi. È emerso che per le puree la capacità di trattenere i liquidi aumenta all'aumentare del tempo del blanching (BL). Le puree BL+H hanno quantità maggiori

di acqua intrappolata o debolmente legata delle puree H+BL, indicando che l'ordine delle operazioni agisce sull'acqua strettamente legata, mentre il tempo di BL agisce sull'acqua debolmente legata. L'effetto delle forze meccaniche sul volume dinamico del CWM è più pronunciato nei campioni BL+H, in quanto le fibre del frutto sono state allentate dal trattamento termico preliminare, mentre nelle puree H+BL il trattamento di omogenizzazione preliminare ha aumentato l' area superficiale dei CWM, ma non il loro volume dinamico (SC).

La terza parte della ricerca (Topic 3) è stata dedicata a determinare l'influenza dello schema di trasformazione sulla composizione in antocianine e composti fenolici delle frazioni liquida (facilmente accessibili) e solida (scarsamente accessibile) dell'omogenato. I composti fenolici dissolti sono stati campionati nelle frazioni liquide separate per gravità naturale o per centrifugazione dai prodotti, mentre i polifenoli associati alle particelle di tessuto sono state estratte con solventi selezionati. Le frazioni liquide e gli estratti sono stati analizzati per i composti fenolici totali (TPC), gli indici di colore e per gli antociani totali e individuali tramite tecniche spettrofotometriche e cromatografiche. La scottatura dei frutti intatti non ne modifica TPC e MAP, tuttavia aumenta il rilascio di TPC dai compartimenti originari del frutto e /o protegge gli stessi dalla degradazione ossidativa durante la successiva omogenizzazione. Ne consegue che TPC e MAP sono maggiori nelle puree BL+H che in quelle H+BL). Inoltre, nelle puree, il blanching preliminare delle bacche (BL+H) determina un maggior rilascio di MAP nella frazione liquida del prodotto mentre il blanching della matrice già omogenata (H+BL) determina un aumento della proporzione di TPC e MAP legati alla frazione solida. L'analisi HPLC-DAD ha indicato che il maggior recupero di composti antocianici nelle puree BL+H ripetto alle H+BL è collegato all'effetto protettivo nei confronti di delfinidine e petunidine del trattamento BL iniziale sulle bacche. Inoltre, negli omogenati, le delfinidine sono principalmete associate al CWM e le malvidine alla fase liquida.

Una parte rilevante dei fenomeni chimico-fisici e biochimici studiati avviene a livello microscopico, fra pareti cellulari e membrane. Perciò l'ultima parte della ricerca (Topic 4) è stata dedicata allo studio della microstruttura dei campioni, separando per sedimentazione i componenti del particolato degli omogenati e analizzando tramite microscopia in fluorescenza le caratteristiche istochimiche sia dei frutti di mirtillo prima e dopo trattamento termico sia del particolato. Nei prodotti BL+H sono presenti cellule singole e la continuità delle membrane e delle pareti è meglio preservata, ma l'ultrastruttura delle pareti cellulari è profondamente alterata, con fibrille di cellulosa "strappate" dalla superficie della parete e un reticolo diffuso di cellulosa fibrillare che incrementa l'area superficiale attiva della matrice. Nei prodotti H+BL sono evidenti agglomerati di cellule più grandi e aggrovigliati nei quali la continuità delle pareti cellulari e delle membrane è persa, producendo un minore volume della fase particolata e una maggiore quantità di materiale citoplasmatico libero.

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0. PREFACE

Increasing the daily intake of fruit and vegetables is considered a priority in Europe, according to the Food-Based Dietary Guidelines (EUFIC REVIEW 01/2012). In fact it has been estimated by the World Health Organization (WHO) that in several EU States the consumption of fruit and vegetables is below the recommended amount of 400 g per day (EUR/06/5062700/BD/2), despite the well-documented benefits of plant food on health. Indeed, data from nutritional studies are increasing which confirm the role of fruit and vegetables in preventing chronic and degenerative diseases and correlate these properties to a synergistic effects on health of specific plant constituents, secondary metabolites and fiber components (Liu, 2013). Thus, in order to improve dietary habits, initiatives have been promoted, also including the development of innovative, healthier and attractive plant-based food products, such as soups and smoothies, containing vegetables and berries.

Highbush blueberry (*Vaccinium corymbosum* L.) fruits are a natural source of phenolic bioactive metabolites, especially anthocyanin molecules, phenolic acids and flavonols, mainly localized into the vacuolar compartments and associated to the epidermal layers fruit and seed (Michalska & Łysiak, 2015). These secondary phenolic metabolites, which exert signaling and protective functions in the plant, seem to modulate similar activities on human cells too (Kennedy, 2014). In fact dietary polyphenols, in synergy with fibers and other plant components, trigger antioxidant and anti-inflammatory pathways in humans, which result in important beneficial effects on health, in particular with respect to chronic diseases (Szajdek & Borowska, 2008).

Processing of blueberries into puréed products would extend the fruit commercial life while contributing to increase the range of attractive and innovative plant-based foods. Blueberry purées are processed without pomace loss and thus potentially rich in all the bioactive compounds which characterize the whole berry. Furthermore, colour and flavour make them valuable and versatile matrices, suitable to be directly consumed or included as ingredients in the increasing market of blended beverages.

Fruit and vegetable liquid-like products are perceived by consumers as healthy and convenience foods, indeed a lack of knowledge still exists on phenomena which link process to functions in these matrices.

Literature data on blueberry processing indicate that antioxidant and sensorial attributes of raw berries could be depleted by extensive mechanical and thermal processing (Howard et al., 2012). In crude blueberry blends, enzymatic activities are responsible for undesirable browning and pigments degradation phenomena (Kader et al., 1997; Skrede et al., 2000; Lee et al., 2002). In fact, due to mechanical tissue breakdown, polyphenol oxidase (PPO), located in the cell cytoplasm, oxidizes chlorogenic acid and consequently anthocyanins, located in the vacuoles, and brown pigments are rapidly developed. Furthermore, stabilizing heat treatments may directly affect anthocyanin structure, damage increasing with magnitude and duration of heating (Brownmiller et al., 2009; Patras et al., 2010). Furthermore, it has been shown that the removal of skin, the main phytochemicals reservoir in blueberry fruits, during juice processing, has a detrimental impact on phenolic profile of berries (Ribera et al., 2010; Lee et al., 2004). On the contrary, blanching pretreatments proved to enhance the quality of several blueberry-based products (Brambilla et al., 2008; Rossi et al, 2003; Sablani et al., 2010). Simultaneous occurrence of enzyme inactivation and tissue swelling is a key-element in blueberry blanching treatments, which increase phenolic stability and phenolics release from the fruit matrix at the same time (Brambilla et al., 2011; Fava et al., 2006).

On the other hand, increasing data are emerging which reveal the importance of microstructal approach to the study of functional and nutritional properties of purée products (Moelants et al, 2014; Parada & Aguilera, 2007; Van Buggenhout et al., 2012). Homogenate products are in fact plant-based dispersions, characterized by a direct involvement of tissue architecture on the quality traits.

Literature data on vegetable-based puréed products indicate that in these matrices, under the effect of thermal and mechanical forces, native plant tissues loose continuity, a new system of solid particles in viscous liquids is shaped and bioactive molecules find a new arrangement all through the liquid and particle phase of the newly-formed system (Anese et al., 2013; Lopez-Sanchez et al., 2015; Svelander et al., 2010). Furthermore, physico-chemical properties of cell wall components are affected by grinding and heating operations, so determining the ability of fibers to sequester/release water and solutes and influencing the fate of dispersed bioactive components consumed through diet (Bayod et al., 2005; Dhingra et al., 2012; Guillon et al., 2000; Padayachee et al., 2013).

Thus, not only the overall content in bioactive molecules, but also the rate of accessible to particle-bound or entrapped constituents are modified through processing, with multiple effects on quality attributes of derived processed suspensions. Hence, both microstructure and rheology concur with biochemical composition in defining the overall quality of a plant food dispersion and all these aspects should be taken into account in the product design process.

To design innovative fruit products, not only rich in potentially active compounds, but actually delivering suitable rheological, sensorial and healthy properties, a deeper comprehension of fruit matrices as dynamic systems is needed. Blueberry-based products of high nutritional and sensorial quality could be developed combining minimal processing design to mild thermal treatments. On the other end, much research is still needed to elucidate the early linkage between mechanical and thermal treatments and phenolic release at the microscale level, which is the first step to get controlled structuring of fruit products tailored on consumer and industry demands.

This PhD thesis investigates the influence of mechanical and thermal treatments on the accessibility of phenolic phytochemicals in blueberry purées, taking into account both the total content in phenolic bioactive compounds and the proportion of particle bound to dissolved constituents. To this aim a processing plan was developed to prepare blueberry-derived homogenate products from individually quick frozen (IQF) fruits. Heat treatments were applied either before or after the homogenization steps by a vacuum-blanching procedure. Hydration properties of the purées were studied by separating the liquid from the particle components and phenolic profile of the fractions was studied. Microstructure of blueberry fruits and homogenate particles was explored by means of fluorescence microscopy.

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1. STATE OF THE ART

1.1 Highbush blueberry

Blueberries (*Vaccinium* spp.) are perennial flowering plants of the Ericaceae family which count more than 400 species of edible fruits and among them are some of the best natural sources of anthocyianin phytochemicals. In Italy, *Vaccinium corymbosum* (L.), also called highbush blueberry, is the most cultivated specie of the *Vaccinium* genus. In last few decades consumers interest for this berry fruit has been growing and thanks to improvements in propagation and cultivation techniques, this crop has spread in suitable areas like Trentino, Piemonte and Lombardia regions.

Blueberries are fleshy indehiscent fruits, they derive from a single epigenous flower, that is a flower characterized by an inferior ovary, hence they are considered by botanist "false berries" *vs* "true berries" which derive from a superior ovary. In false berries, basal parts of the flower develop with the ovary forming the pulpy fruit, as evidenced in blueberries by the persistent calix (remains of stamen, styles and sepals) visible at the opposite side of the stem (Figure 1.1). Hence, in blueberries, the edible portion is not the wall of the ovary but of the floral component (thalamus).

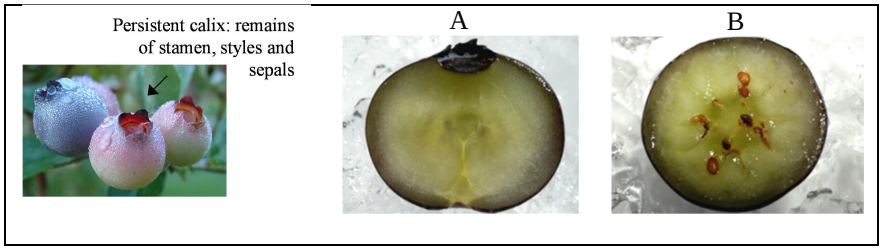

Figure 1.1: Highbush blueberry fruits: longitudinal (A) and mid-cross (B) sections.

1.1.1 Histochemical description

Blueberry fruits are characterized by a whitish waxy surface consisting of epicuticular waxes arranged in amorphous layers or crystalline platelets or rodlets. Follow in the order the cuticle layer, consisting of cutin and intracuticular waxes (5 μm thick approx.), a pectic/cellulosic layer, which lean on epidermal cells, and few layers of epidermal cells where anthocyanin pigments are found, stored in the vacuolar compartments (epidermal and hypodermal pigmented cells) (Fava et al, 2006; Sapers & Phyllips, 1985). A ring of vascular bundles separate epidermal layers to the inner flesh of the fruits while at the center there are placentas and numerous seed. Stone cells are distributed below the epidermis and in the flesh, contributing to the fruit firmness (Allan-Woytas et al, 2001). Structure of surface waxes, skin thickness, shape, size and abundance of stone-cells as well as arrangement of thick walled vascular tissues may affect texture, firmness, anthocyanin accumulation and degree of pigments bleeding during handling, hence they are relevant for technological, nutritional and storage attitudes of the fruits (Allan-Wojtas et al, 2001). These histochemical traits provide protection against adverse external biotic and abiotic factors, hence they are cultivar-dependent parameters, but their expression is strictly affected by environmental conditions (Konarska 2015).

1.1.2 Fruit composition

Blueberries generally consist of water (84%), carbohydrates (9.7%), proteins (0.6%) and fat (0.4%). Fresh blueberry fruits are a good source of dietary fiber (3%–3.5% of fruit weight), they contain a good amount of minerals like potassium (77 mg/100g) and manganese (0.336 mg/100g) and are characterized by a moderate content of vitamin C (100 g of blueberries provide 10 mg of ascorbic acid, on average) (Michalska & Łysiak, 2015**;** Prior et al., 1998). Above all, blueberries are a rich source of phenolic metabolites, notably anthocyanins, flavanols and hydroxycinnamates (Kalt et al, 1999; Prior et al, 2001).

The total content of polyphenols in blueberries ranges from 48 up to 304 mg/100 g of fresh fruit weight (Ehlenfeldt & Prior, 2001) while anthocyanin content of blueberries has been reported to range from 25 up to 495 mg/100 g of fruit (Mazza & Miniati, 1993). Accumulation of phenolic metabolites is cultivar-dependent even if it is deeply influenced by fruit size, ripening stage, and environmental conditions (Prior et al, 1998; Howard et al. 2003). It is the complexity of anthocyanin profile that characterizes these berries, which contain five main anthocyanidins (malvidin, delphinidin, petunidin, cyanidin and peonidin) combined to three sugar moieties (glucose, galactose, arabinose).

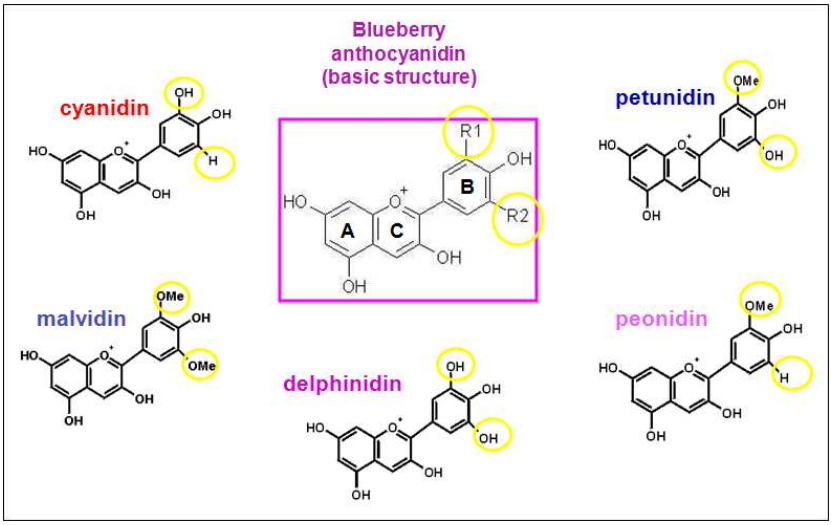

Figure 1.2: Chemical structure of blueberry anthocyanidins

The principal anthocyanidins differ in number and position of the hydroxyl and /or methyl ether groups attached on 3', 5' in ring B. With the increase of attached hydroxyl groups, the visible colour of entire molecule shift from orange to violet (Figure 1.2). Malvidin and delphinidin are the major anthocyanidin components of blueberries and constitute 75% approx. of all identified anthocyanins. As a function of the rings orientation, degree/position of hydroxyl groups on the B ring and degree/position of methoxyl groups, anthocyanin compounds are characterized by specific chelating and antioxidant capacities, which are at the basis of their bioactivity (Bravo, 1998). In particular, the antioxidant potential increases with the number of hydroxyls, thus delphinidin-3-glucoside and cyanidin-3-glucoside exhibiting greater reactivity compared to petunidin-3-glucoside and malvidin-3-glucoside (Miguel, 2011).

1.2 Biological activities of phenolic compounds

Phenolics in raw fruit tissues are mainly localized in cell vacuoles or associated with cell wall structures. In particular, anthocyanin compounds characterizing blueberry fruits are confined and protected by enzymatic degradations into the cell vacuoles of few epidermal layers underneath cuticle waxes. Polyphenols are stress-responsive compounds, involved in photo protection phenomena, which are able to regulate plant-environment interactions by activating network of events (secondary antioxidant system). In native plant tissues these multiple functions are regulated through location within different cell compartments (vacuoles, cell walls) and governed through dedicated transport, secretion and accumulation systems (Agati et al, 2012).

Biological activity of dietary anthocyanins in humans is somehow analogous to the bioactivity that these molecules exert in the original plant environment. Berry polyphenols in fact, by activating antioxidant and non-antioxidant signaling pathways similar to the one activated in plants, contrast stress-induced damages, which in humans can predispose to the development of chronic diseases (Beattie et al, 2005) (Figure 1.3). Hence the ACNs bioactivities which have been documented by *in vitro* and *in vivo* studies, which include anti-inflammatory properties, improvement of lipid profiles, modulation of detoxifying enzymes, and reduction of blood pressure and platelet aggregation (Del Rio et al., 2010; Wang and Stoner, 2008).

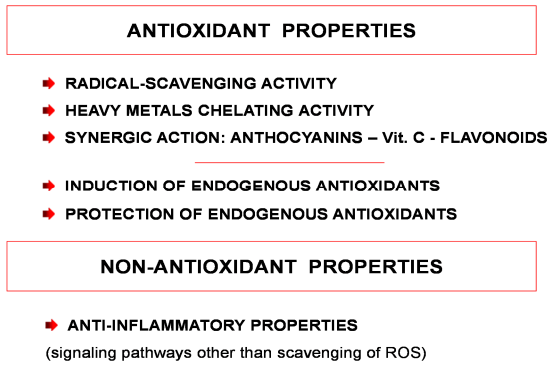

Figure 1.3: Mechanisms for biological activity of dietary anthocyanins

1.3. Effect of processing on blueberries

Quality traits of blueberry fruits are strictly related to the histochemical features of their compact and waxy epidermal layers of cells, which affect their storage, handling and processing attitudes. Hence, microstructural investigations on blueberry fruits in literature are mainly addressed to their peripheral layers of cells.

Fava et al. (2006) analysed microstructural alterations induced by blanching, freezing-thawing and ultrasound on epidermal cells of blueberry fruits, by means of light and atomic force microscopy. They reported that blanching of blueberries in saturated vapour produced melting of the epicuticular waxes, swelling of epidermal and subepidermal cells walls and partial plasmolysis. Cellulose fibrillary network of epidermal cell walls appeared loosened under atomic force microscopy, with gaps and ruptures occurring between xyloglucan chains. These

Pre-Treatment Method		Influence on the Product Quality
Storage	Modified atmosphere	Total phenolics (↑) Antioxidant capacity (–) Vitamin C (–)
Thermal	Freezing	Total phenolics (↑) Total anthocyanins (–) Delphinidin glucoside (↑)
	Blanching	Total anthocyanins (↑) Total anthocyanins (–) Antioxidant capacity by ORAC (↓)
Mechanical	Cutting (halves/quarters)	
	Scarification	
	Abrasive skin removal	Total phenolics (↓) Vitamin C (↓)
Chemical	Chemical substances	Organoleptic properties (↓)
	Natural substances	Total phenolics (–) Total anthocyanins (–) Antioxidant capacity (–)
Processing Methods		**Influence on the Product Quality**
Juicing		Total monomeric anthocyanins (↓)
Dehydration	Osmotic dehydration	Total phenolics (↓) Total anthocyanins (↓)
	Freeze-drying	Vitamins A, C and niacin (↑) *,*** Polyphenols (ellagic acids, quercetin, naringin, kaempferol) (↑) *,** Antioxidant capacity (↑) *,**
	Hot air drying	Total phenolics (↓) *** Total anthocyanins (↓) *** Antioxidant capacity (↓) ***
	Fluidized bed drying	Total phenolics (↓) Total anthocyanins (↓)
	Heat pump drying	Total monomeric anthocyanins (↑)
	Vacuum drying	Volatile compounds (↑) ** Total phenolics (↑) ** Total anthocyanins (↑) **
	Radiant zone drying	Total phenolics (–) *** Total anthocyanins (–) *** 13 Identified anthocyanins (–) ***

–, no influence; ↑, increase in the content/properties; ↓, decrease in the content/properties; * compared to microwave vacuum drying; ** compared to hot air drying; *** compared to freeze-drying.

Figure 1.4: Changes in blueberry product quality in terms of selected bioactive compounds and quality properties depending on a pre-treatment and the processing method. [Adapted from Michalska & Łysiak, 2015].

phenomena were related to the increased thickness of the outer tangential cell walls in the blanched fruits. Besides, inner edible pulp of blanched berries appeared well preserved. In ultrasound-treated berries, packing of the fibrillary network increased and thickness of epidermal walls decreased, with consequent formation of cavities in the epicarp of fruits which increased their permeability. Finally, domestic freezing and thawing produced the disruption of plasma membranes with consequent fruit softening, due to extracellular ice formation. An increase in the permeability of blueberry waxy skin is desired in the case of pre-treatments to osmotic dehydration (Giovanelli et al., 2012). In this context also liquid nitrogen treatments were tested and their influence on the microstructure of blueberry epidermal layers was studied (Ketata et al., 2013). Also in this case microscopic study evidenced dewaxing phenomena and epidermal micro-fissures with consequent increase in the permeability of the fruits. Figure 1.4

summarizes the effects of storage, thermal, mechanical and chemical pretreatments as well as of juicing and dehydration processing methods on selected bioactive compounds

1.4 Phenolic accessibility

Bioactive molecules exert their functions when they are present at the right concentration in the place of action (Fig. 1.5). In plants, several transport system deliver for phenolic secondary metabolites are active to this aim (Kennedy, 2014). In humans few data are available which describe actual pathways for phenolic delivering to the place of action.

Literature data indicate that absorption of anthocyanins begins in the stomach where an active transporter protein is present. In the small intestine pH conditions (pH=7) are not favourable for these compounds (pH optimal for ACN=1) but beta glucosidase of epithelial cells may cleave the sugar moiety, producing a smaller and more hydrophobic aglycone, prone to passive diffusion. The anthocyanins which have not been absorbed travel through the intestine and meet the gut microflora which cleave the glycosidic linkage and break down anthocyanins into phenolic acids. *In vivo* studies indicate that the overall absorption of anthocyanins during gastrointestinal digestion is very low (0.02-0.2%) (He et al., 2009).

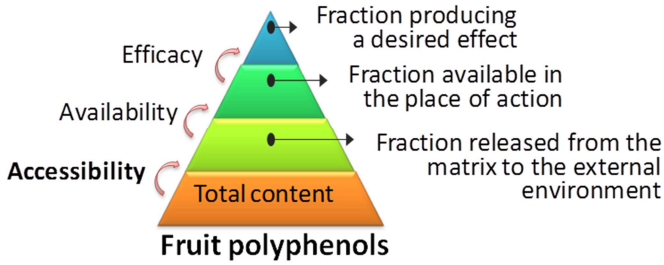

Figure 1.5: Accessibility of a phytochemical compound in a food matrix as first step to its availability and effectiveness.

On the other hand, it has been explained that both physiologically extractable phenolics (released from the food matrix during gastrointestinal digestion) and physiologically non extractable phenolics (not released from the food matrix during gastrointestinal digestion because associated to indigestible cell walls) can be biologically relevant. In fact they are fermented by the colonic microflora which release from the fibers low molecular weight phenolic compounds (Ross, 2014).

Hence, it has to be considered that indigestible cell wall materials are relevant for phenolic bioactivities because they can hinder the release of phenolics but can also protect and enhance their transportation to the place of action (Mazza & Kay, 2008; Williamson & Clifford, 2010; Cheynier, 2005).

In intact fruit systems the mobility of phenolic compounds is actively governed throughout the hierarchically organized tissue environment (Parada & Aguillera, 2007). Phenolic compounds can be found within the cell cytoplasm and vacuolar sap either in free solution or linked to protein matrix forming inclusions, or they can be associated with cell walls components,

entrapped into cavities or forming complexes through hydrophilic and hydrophobic interactions (Pinelo et al., 2006). In homogenate products native tissue environment is destroyed, a new system of solid particles in viscous liquids is shaped under thermal and mechanical forces and phenolic molecules find a new arrangement all through the liquid and particle phases of this newly-formed system. Polyphenols released from the processed tissue compartments and not affected by depletion and oxidation phenomena would flow together into the liquid phase of the homogenates, while immobilized polyphenols as far as newly bound or entrapped phenolics would remain associated to the homogenate dispersed phase.

In the case of anthocyanin compounds, it was evidenced that a preliminary berry-blanching step introduced in the processing-line used for blueberry purées determined the diffusion of pigments from epidermal layers into the centre of the fruit, enriching the liquid fraction of the products (Brambilla et al., 2011). It was shown that the absorption of anthocyanin in human plasma after the consumption of blueberry purées was enhanced by the berry blanching pre-treatment, as reported in a parallel nutritional study (Del Bo' et al., 2012) as well as the linkage between the intake of an anthocyanin-enriched diet and the improvement of cell antioxidant defence against DNA damage in humans (Del Bo' et al., 2013).

1.4.1. Cell wall polysaccharide-Phenolic interactions

Potential binding between anthocyanins/phenolic acids and cell wall materials can occur when these metabolites are released from the plant cell vacuoles due to processing or mastication (Padayachee et al., 2013). Based on literature data in apples these interaction seem to be influenced by the pore size and matrix organization and hydrophobic/hydrophilic regions of cell walls but also selective interactions between pectins/cellulose and individual polyphenols can occur (Renard et al., 2001; Le Bourvellec et al., 2004). Studies on binding of polyphenols in purple carrots and plant cell wall analogues (Padayachee et al., 2012a; Padayachee et al., 2012b; Padayachee et al., 2013) revealed that both cellulose and pectins can interact with phenolic acids and anthocyanins, binding increasing with the time of contact between anthocyanins and cell wall materials, following a rapid initial binding phase (direct anthocyanin interactions with cellulose and pectins) and a slower subsequent binding (stacking effect, anthocyanins interact with already bound anthocyanins).

1.5. Structural design of plant-based food: plant food dispersions

One definition of plant food is *"a watery solution of low molecular weight species, mainly sugars, salts and organic acids and of high molecular weight hydrocolloids, contained in a water insoluble cellular matrix of macromolecules, mostly carbohydrates, including insoluble pectin substances, hemicellulose and proteins"* (Maltini et al., 2003).

In intact fruit systems this "water insoluble cellular matrix" consists in a hierarchically organized tissue environment, while in plant-based foods, solutes are dispersed in a new microstructure produced by processing (Parada & Aguilera, 2007). Microstructure can be defined as the spatial arrangement of constituents at the micro−scale level, that in plant food is the level of tissue cell environment, including cell walls and membranes, fibers, granules, polymer clusters, droplets, bubbles and crystals (Fig. 1.6).

In both native and processed food products of plant origin, bioactive phytochemicals inevitably interact with water and cell wall materials hence the "structural design" of plant-based foods is essentially the design of cell wall components in a serum phase.

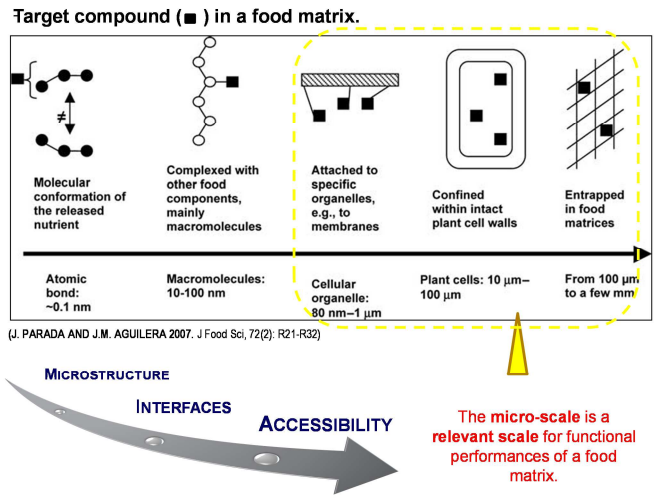

Figure1.6: Microscale phenomena in plant-food. [Adapted from Parada & Aguilera, 2007].

In these last few years the microstructure of plant food dispersions have been the subject of several investigations, due to two main orders of facts. Firstly, the relevance on the market of fluid-like commodities derived from vegetables and fruits, which include soups, pastes, sauces and purée products. Secondly, plant food dispersions can be considered as model systems to investigate the "endogenous structuring potential" of fruit and vegetables (Van Buggenhout et al., 2012), potential at the basis of the development of innovative healthier plant-food products. Increasing literature is available addressed to the study of vegetable–based homogenate products, in particular derived from tomato and carrots (Lopez-Sanchez et al., 2011a, b; Lopez-Sanchez et al., 2015; Moelants et al., 2014a; Christiaens et al., 2012).

Vegetable-based suspensions consist in a particle fraction in a continuous serum phase, shaped under de-structuring (blending, grinding, sieving, homogenization) and stabilizing (heating, high-pressure) unit operations (Fig. 1.7). Both plant-related and process-related factors affect the microstructure of particles in suspension (Moelants et al., 2014b).

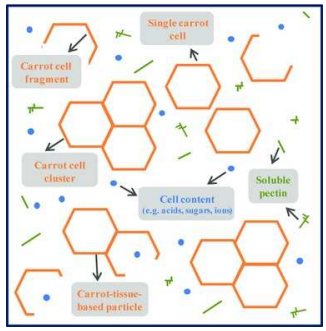

Figure 1.7: A schematic representation of the composition of plant-tissue-based food suspensions. (Source: Moelants et al., 2014b; from Hendrickx, 2014)

With respect to plant-related factors, it has to be considered that plant cells are supported by a fibrillary cellulosic network (cell wall or "exoskeleton") and are glued together by an amorphous pectin matrix (middle lamella) (Kunzek et al., 1999; Waldron et al., 2003). The edible portion of fruit and vegetables mainly consists of parenchymatic cells, which are typically thin-walled but can vary in size (50-500 μm) and shape (isodiametric or elongated) depending on the plant source and the location within the organ (epidermal *vs* inner portion). Any change in one of these parameters related to plant source, variety, maturity or environmental conditions, will affect also the morphology of tissue particles in mechanically processed tissues (Moelants et al., 2014).

When stabilising thermal treatments are applied, a range of chemical and physical events are triggered which result in: (i) loss of turgor, due to disruption of the plasmalemma and free diffusion of cytoplasmic content, (ii) cell softening/separation, due to beta-elimination of pectins polysaccharides or acidic hydrolysis of glycosidic bonds, (iii) swelling of cell walls, maybe due to thermal degradation of polymers stabilizing cellulose fibrils. Furthermore, the activity of pectinolytic enzymes can be modulated by controlling processing temperature, thus increasing or reducing the product firmness (Moelants et al., 2014b).

Under the effect of mechanical forces, the weakest links within tissue structures fail, this resulting in cell rupture or cell separation depending on the strength of cell-cell adhesion along middle lamella. In fact, microscopy investigations on carrots, broccoli and tomato dispersions revealed that classes of plant particles could be obtained, different in size, shape, and surface properties, starting from the same plant material but acting on order and intensity of processing operations (Lopez-Sanchez et al., 2011a, b; Lopez-Sanchez et al., 2015; Moelants et al., 2014a; Christiaens et al., 2012). In particular, large cell clusters with rough surface and broken cells are mainly recovered when blending is applied on native plant tissues, while tissue fragments small in size, with smooth surface and individual intact cells are recovered when thermally treated tissues are blended.

Hence the microstructure of particles in plant-food dispersions is related firstly to morphometric and histochemical features of the native plant materials, secondly to physical-chemical properties of the matrix as determined by enzymatic/thermal pre-treatments, and finally by the intensity of mechanical disruption treatments.

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2. **AIMS OF THE STUDY**

The general aim of this PhD project was to investigate the influence of mechanical and thermal treatments on phenolic-delivering properties of blueberry-derived purées, taking into account both the total content in phenolic bioactive compounds and the proportion of particle bound to dissolved phenolic constituents.

A complementary physical/chemical, microstructural and biochemical approach was applied. After having drawn up a processing plan for producing blueberry-derived homogenates by combining mechanical and thermal treatments of increasing intensities with heat treatments planned either before or after the homogenization steps, the effects of the order and duration of processing operations on chemical/physical and hydration properties of the products were considered. Then the influence of processing on the composition in anthocyanin and phenolic molecules of the liquid (easily accessible) and particle (scarcely accessible) fractions of the homogenate systems was assessed and in the end the microstructure of the samples was studied.

3. TOPIC 1

Preparation of blueberry-derived homogenates

In this study the influence of mechanical and thermal treatments on phenolics accessibility was explored in the particle-liquid system of minimally-processed blueberry purées.
In the first part of this research, a processing plan was developed which combined heating and blending operations to produce puréed products from the fruits.
Heat treatments were planned either before or after the homogenization step and to this aim a vacuum-blanching procedure was implemented in order to blanch either fruit or puréed samples and avoid liquid dispersion by leeching. Preliminary trials were carried out to assess thermal inactivation of polyphenol oxidase (PPO). Experimental sets of samples were produced by combining two blending times (5s, 15s) and four blanching times (80s, 160s, 240s, 320s) and the purée-processing was replicated three times in different days.

3.1. Materials and methods

3.1.1. Experimental set up

The purpose of this research was to investigate the influence of mechanical and thermal treatments on phenolic-delivering properties of blueberry derived matrices. To this aim a processing plan was drawn up to prepare blueberry-derived homogenate products from individually quick frozen (IQF) fruits. Heat treatments were planned either before or after the homogenization step and a vacuum-blanching procedure was developed to this aim. Experimental variables included blanching (BL) time, homogenization (H) time and operation order (BL+H or H+BL) according to the flow-sheet reported in Figure 3.1. The whole processing workflow was replicated three times in three subsequent days. Chemical/physical and microstructural properties of experimental samples as far as anthocyanin and phenolic composition of the particle and liquid components were analyzed to link processing variables to phenolic release in blueberry homogenate products.

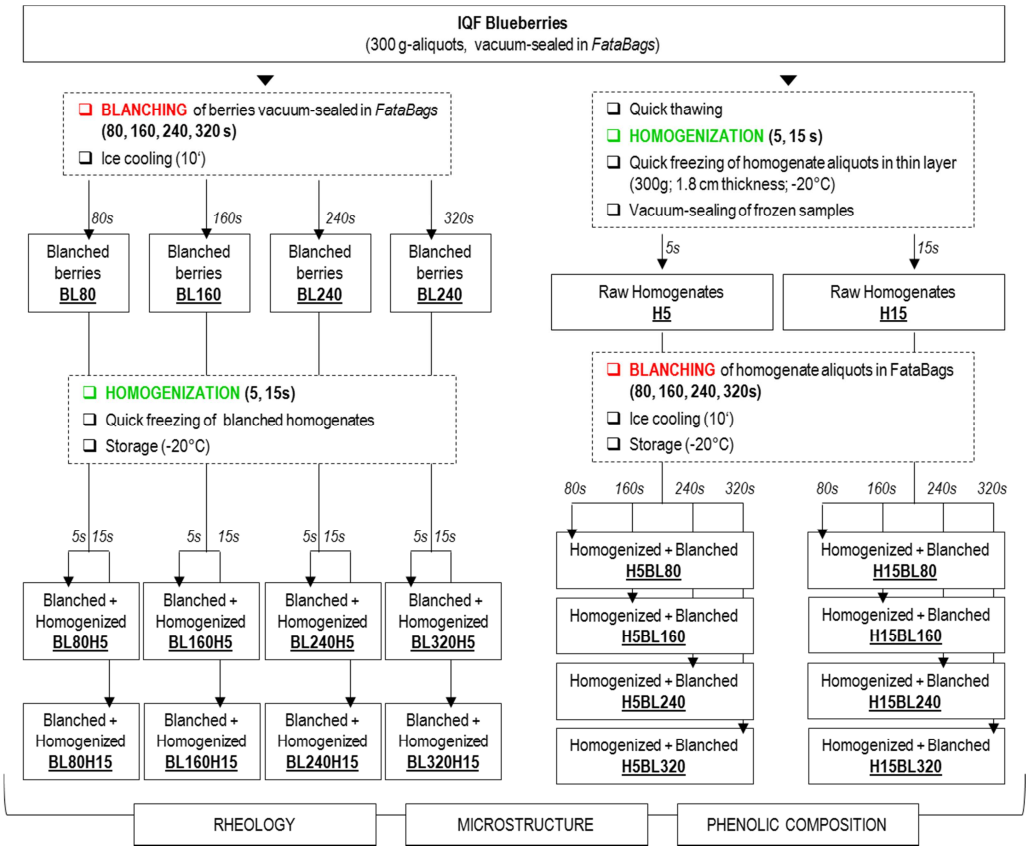

Figure 3.1: Flow-sheet of the processing of blueberry-derived homogenates with products coding.

3.1.2. Plant material

Highbush blueberry fruits (*Vaccinium corymbosum* L. "Brigitta"), harvested at market maturity in the Valtellina district, were cleaned, individually quick frozen in a tunnel (Thermolab, Codogno, Italy) operating at −50 °C air temperature and 4.5 m/s air speed and stored at −20°C till processing. Research was carried out on a batch of 30 kg of berries.

3.1.3. Thermal treatments

All thermal treatments were performed in boiling water (20 L) by individually blanching 300 g aliquots of berry/homogenate products, previously sealed under partial vacuum (Vacuum Pump S.p.A., Como, Italy) in special bags for high degrees vacuum cooking (*Fata Bags*, Decorfood, Italy). Frozen blueberries were sealed in a single layer of fruits and raw homogenates in a frozen-moulded tile shape (210×140 mm; 1.8 cm thick) in order to ensure a more uniform heat treatment for both types of products (Figure 3.2). After blanching treatment, samples were promptly cooled in an ice water bath for 10 min before undergoing further processing. Blanching times were chosen on the basis of results on thermal inactivation of blueberry polyphenol oxidase (PPO) obtained in the preliminary PPO inactivation assay.

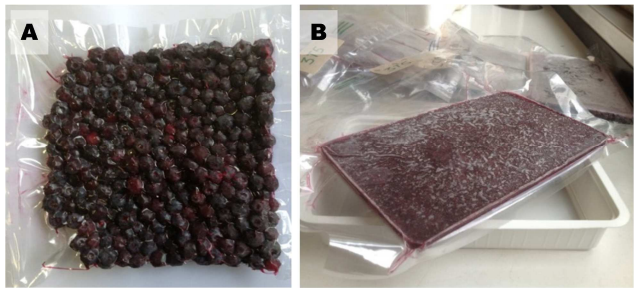

Figure 3.2: Vacuum sealed blueberry fruits (A) and homogenate (B) samples

3.1.3.1 Preliminary PPO inactivation assay

Polyphenol Oxidase (PPO) is the main enzyme involved in the enzymatic browning of blueberry products, it is bound to the thylakoid membranes and it is characterized by high inactivation temperatures. A preliminary assay on residual PPO activity of homogenates after a blanching treatment of 40 s, 80 s, 120 s and 160 s was carried out according to Kader et al. (1997) with few modifications. Extracts were prepared by diluting 5 g of homogenate products with 25 g of McIlvaine buffer pH 4, containing 4% polyvinylpolypyrolidone (PVP), centrifuging at 15.000 × *g* for 20 min at 4 °C and filtering the supernatant through a 0.45 µm filter. PVP was added to remove from the samples phenolic compounds which could interfere with the assay and its best concentration, in the 1-5% range, was firstly assessed. For the PPO assay, 850 µl of McIlvaine buffer pH 4, 100 µl of 1 mM chlorogenic acid in the same buffer and 50 µl of the homogenate extract were mixed and the absorbance increase at 400 nm (25 °C) was registered for 15 min by a spectrophotometer (Jasco, Easton, MD). In our conditions, a minimum water-blanching time of 160 s was necessary to cut by 90 % approx initial PPO activity (Fig. 3.3).

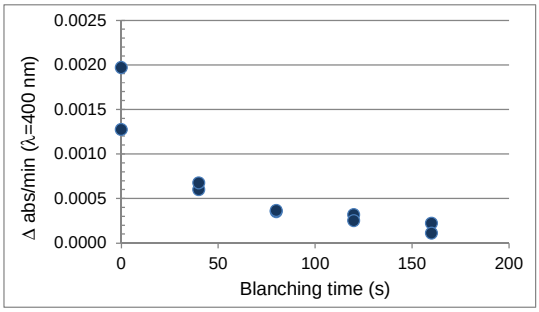

Figure 3.3: Activity of blueberry PPO as a function of fruit blanching time, measured on a chlorogenic acid substrate, at pH 4**.**

3.1.4. Mechanical treatments

Homogenization was performed using a two speed Waring 7011 HS blender (Waring Laboratory and Science, Torrington, CT) equipped with 1 L-capacity stainless steel container. IQF berry samples, just thawed in a water bath (1 h; room temperature) and BL berries, cooled in ice water, were homogenized at low speed for 5 s (H5) or 15 s (H15). BL homogenates were divided into aliquots and stored at −20 °C for subsequent analysis while raw homogenates (300 g aliquots) were frozen at −20 °C in plastic food trays (210×140 mm) for 48 h and then vacuum sealed in *FataBags* (Figure 3.2 B) for subsequent blanching treatments.

3.2. Results

3.2.1 Experimental set of samples

Sets of processed fruit and homogenate samples were obtained from IQF blueberries by combining blanching time (0, 80, 160, 240 and 320 s), homogenization time (5 and 15s) and operation order (BL+H or H+BL). Hence, each set of samples consisted of untreated (1) and thermally treated (4) whole berry samples and untreated (2) and thermally treated (8+8) homogenate products, for a total of 23 different samples and three independent replicates (69 specimens) as reported in Figure 3.4. All the samples were stored at −20°C vacuum sealed in *FataBags* (IQF and BL berries, H and HBL homogenates) or in plastic tubes (BLH homogenates).

	H = 0 s	H = 5 s		H = 15 s			Samples coding:				
BL = 0 s	Berries	Purées		Purées			BL0	H5	H5	H15	H15
BL = 8 0 s	Berries	↑Purées	↓Purées	↑Purées	↓Purées	=	BL80	BL80H5	H5BL80	BL80H15	H15BL80
BL = 160 s	Berries	↑Purées	↓Purées	↑Purées	↓Purées		BL160	BL160H5	H5BL160	BL160H15	H15BL160
BL = 24 0 s	Berries	↑Purées	↓Purées	↑Purées	↓Purées		BL240	BL240H5	H5BL240	BL240H15	H15BL240
BL = 320 s	Berries	↑Purées	↓Purées	↑Purées	↓Purées		BL320	BL320H5	H5BL320	BL320H15	H15BL320

Figure 3.4: Experimental sets of samples generated from the combination of processing variables: homogenization (H) time, blanching (BL) time and operation order (↑=BL+H; ↓=H+BL).

3.3. References

Kader F et al., 1997, Mechanism of browning in fresh highbush blueberry Fruit (*Vaccinium corymbosum* L). Partial purification and characterisation of blueberry polyphenol oxidase. *J Sci Food Agric* **73**:513–516.

4. TOPIC 2

Study of hydration properties of blueberry-derived homogenate products

This part studied the influence of blanching and homogenization steps on the physicochemical and functional properties of blueberry-derived homogenates. In particular it was examined the effect of the order and duration of treatments on hydration properties of the homogenate samples. Liquid retention capacity was tested and particle swelling of all the samples were measured. The strength by which homogenate products retain their liquid fraction was investigated by measuring the amount of serum released from homogenate samples (expressible liquid fraction) with specific geometry and weight under the influence of different and increasing driving forces. Two protocols were developed for measuring the amount of liquid lost from homogenate samples under gravity (G, free or gravitational liquid fractions) by the aid of net filter devices. The amount of liquid released under the combined effect of gravity and weak capillary suction force was measured by a filter paper method (FP, gravitational+capillary liquid fraction), while the sum of free and entrapped or loosely-bound liquid fractions was determined under centrifugal force (C, enhanced gravity or centrifuge liquid fraction). Furthermore, the volume at maximum hydration of particle components decanted in excess of water was measured and compared among samples (swelling capacity, SC). A measure of water activity (a_w) was provided and main quality parameters were assessed.

4.1. Material and methods

Frozen stored homogenates (10-g and 30-g aliquots) were quickly thawed in a water bath at 22 °C just prior to analysis.

4.1.1. Hydration properties

4.1.1.1. Gravitational liquid fraction: filter-washer method

A rubber filter washer (17.5 mm ID × 24 mm OD × 3.2 mm thickness) (Figure 4.1) was placed in the center of a 10-mm paper disk (Whatman) and a fixed amount (0.5 g approx.) of homogenate product was gently distributed over the pre-weighted net filter device.

Figure 4.1: Filter-washer devices for gravitational liquid determination.

The weight of each homogenate-filled filter was registered before and after 1 h at room temperature and the amount of liquid lost by gravity from the homogenates was measured by difference and expressed as percentage to initial homogenate weight, according to Eq. 4.1:

$$G1\ (\%) = \frac{Wi - Wf}{W0.5} \times 100 \qquad (4.1)$$

where: G1 (%) is the gravitational liquid fraction measured by filter-washer method, W0.5 the initial homogenate weight, Wi and Wf are the weights of the homogenate-filled filter registered at the beginning and at the end of the separation process. Each sample was analysed in duplicate.

4.1.1.2. Gravitational liquid fraction: polyester-mesh method

A fixed amount (20 g approx) of homogenate product was placed in a plastic funnel (50 mm top diameter) previously lined with a polyester screen mesh (0.3±0.01 mm hole size) and positioned within a 12-ml test tube. The weight of the homogenate-filled test tube was registered before and after 1 h at room temperature and the amount of liquid lost by gravity from the homogenate was measured by difference and expressed as percentage to the initial homogenate weight, according to Eq. 4.2:

$$G2\ (\%) = \frac{Wi - Wf}{W20} \times 100 \qquad (4.2)$$

where: G2 (%) is the gravitational liquid fraction measured by polyester-mesh method, W20 the initial homogenate weight, Wi and Wf are the weights of the test tube registered at the

beginning and at the end of the separation process. Each sample was analyzed in duplicate and the liquid fractions were collected and stored at −20 °C until further analysis.

4.1.1.3. Gravity+capillary liquid fraction: filter-paper method

A filter paper press method, usually applied for measuring expressible liquid in meat products (Van Oeckel et al., 1999), has been here adapted for homogenate products. A fixed amount (0.5 g approx.) of homogenate product was distributed inside a steel flat washer (10 mm ID × 19 mm OD × 2 mm thickness) (Figure 4.2), previously placed at the center of a filter paper disk (Whatman No. 44, 90 mm diameter), leaning on a Petri plate. The flat washer device was introduced to ensure constant geometry to the homogenate samples.

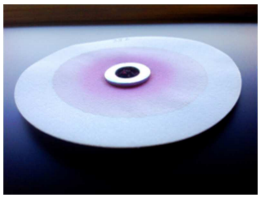

Figure 4.2: Steel flat washerand liquid infiltrated area on a filter-paper disk.

After 1 h at room temperature, minimun and maximum diameters of the liquid infiltrated area were registered by a caliper (EMA-15 DIGI-Kanon, Nakamura Mfg. Co., Ltd, Japan). The amount of liquid released from the homogenate to the filter-paper under the combined effect of gravity and weak capillary suction force was measured indirectly by computing the average infiltrated area according to Eq. 4.3:

$$FP\ (mm^2g^{-1}) = \frac{Ap}{W0.5} \qquad (4.3)$$

where: FP (mm^2g^{-1}) is the filter-paper liquid fraction, A_p the average infiltrated paper area, $W_{0.5}$ the initial homogenate weight. Trials were performed in a glass box to prevent evaporation and 6 replicates/sample were carried out.

4.1.1.4. Enhanced gravity liquid fraction: centrifuge method

A fixed amount (20 g approx.) of homogenate product was placed in 30-ml tubes and centrifuged at 16,000 × *g* for 20 min at 4 °C (Heraeus Biofuge Stratos, Kendro, Germany). The supernatant was poured in 15-ml plastic tubes, its weight was registered and it was expressed as percentage to initial homogenate weight according to Eq. 4.4:

$$C1\ (\%) = \frac{Wc}{W20} \times 100 \qquad (4.4)$$

where: C1 (%) is the liquid fraction expressed under centrifugal force from homogenate samples, W_c the supernatant weight and W_{20} the initial homogenate weight. Each sample was analyzed in duplicate and both the liquid fractions and the corresponding pellets were collected and stored at −20 °C until further analysis.

4.1.1.5. Swelling capacity

Swelling capacity (SC) was determined according Robertson et al. (2000) with some modifications. One gram of homogenate product was weighed in a 30-ml glass tube (ID 15 mm) and 25 ml of distilled water were added. Diluted samples were mixed on a Vortex for 10 s and after 5 min they were mixed up-and-down ten times and allowed to settle for 72 h, until sediment level became constant in all the samples. The test was performed under controlled temperature conditions, in a thermostatic chamber at 4 °C, in order to not influence the settling rate as well as to reduce enzymatic activity. Height of decanted sediment was measured by a digital caliper (EMA-15 DIGI-Kanon, Nakamura Mfg. Co., Ltd, Japan), bed volume occupied by decanted particles was calculated and swelling capacity of the homogenate product was computed according to Eq. 4.5:

$$SC\ (mm^3/g) = \frac{Bv}{Wh} \qquad (4.5)$$

where; SC is the swelling capacity, Bv the bed volume (mm^3) and Wh the initial homogenate sample weight (g). Settling test was repeated four times for each sample.

4.1.1.6. Water activity

Water activity (a_w) was measured with a *AquaLab* (Decagon Devices, Pulman, WA, USA) water activity meter, model 4TE-Dew Point mode. Homogenate aliquots (3 g) were preliminarily allowed to stand for 30 min at room temperature in disposable sample cups sealed by a lid. For the measure of a_w, temperature difference between the sample and the lid was set at +0.67 °C, default temperature was set at 23 °C and temperature equilibration before measurements was set at ±0.5 °C. Custom mode of reading was chosen, which requires the instrument to display the value of the final test after four consecutive tests be within +/− 0.0001 a_w. Three repetitions were planned for each sample and each repetition was read once.

4.1.2. Quality parameters

Soluble solids content (SSC, %) was determined using an automatic refractometer (RFM81, Bellingham-Stanley Ltd, UK) and titratable acidity (TA, meq/100g) was determined by titrating 5 g of homogenate sample plus 50 mL of distilled water with 0.1 N NaOH to pH 8.0. Dry matter (DM, %) was determined by drying approximately 5 g of sample in a forced-air oven (WTB Binder, Tuttlingen, Germany) at 80˚C until a constant weight was obtained. All determinations were carried out in duplicate.

4.1.3. Statistical analysis

Statistical analysis was performed using Statgraphics version 7 (Manugistic Inc, Graphics Software Systems, Rockville, MD, USA) software. Data were processed by multifactor analysis of variance (ANOVA) considering blanching (BL) time, homogenization (H) time, operation order and their interactions as source of variation. Furthermore one-way ANOVA was used to study: (a) the influence of BL-time within the same product, (b) the influence of processing order within the same H-time, (c) the influence of H-time within the same processing order, (d) the influence of the product. Means were compared by Tukey's test at $P \leq 0.05$.

4.2. Results and discussion

4.2.1. Influence of processing variables on main quality attributes of blueberry fruits and puréed products

Fruits

"Brigitta" (*Vaccinium corymbosum* L.) is a mid−late season blueberry cultivar, widely cultivated in the Valtellina district because of the good plant vigor and fruit quality attributes. Fruits from this cultivar are medium−large in size, firm, sweet/tangy and considered appropriated for long storage (Fondazione Fojanini).

IQF blueberry fruits used in this research were characterized by high titratable acidity values (TA), balanced by an elevated content in soluble solids (SSC), according to the quality traits of this variety, with an average dry matter (DM) content of 15.65% and pH value of 3.15 (Figure 4.3).

Figure 4.3: Dry matter (DM), titratable acidity (TA), soluble solids (SS) and pH of "Brigitta" blueberries. (Values are means ± SE; n=6)

The blanching treatments carried out on IQF fruits vacuum sealed in plastic bags did not significantly affect DM and pH of the berries (Table 4.1), while, after an initial decrease with mild BL treatments (BL80), an increase with BL-time was registered for TA and SSC (Table 4.1, Figure 4.4). Besides, homogenization treatments did not affect physicochemical parameters of the berries, unless TA which increased (+12.8% approx.) in extensively blended berries (H15) (Table 4.1).

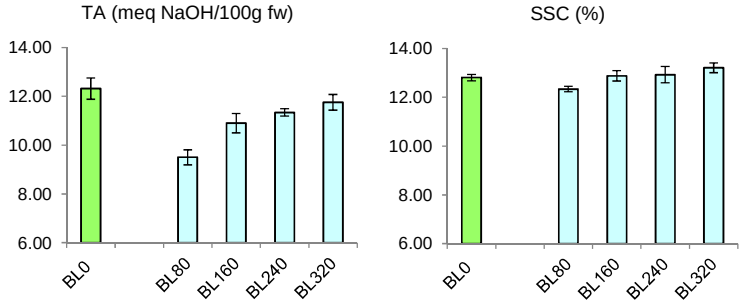

Figure 4.4: Titratable acidity (TA) and soluble solids content (SSC) of blueberry fruits as a function of blanching time (0, 80, 160, 240 and 320s). (Average of the replicates ± std error).

Purées

TA and pH of blueberry purées were not or scarcely influenced by the processing factors as well as by their interactions (Table 4.2). On the contrary, SSC and, to a less extent, DM were mainly influenced by blanching time and secondly by the operation order (Table 4.2). however,

the effect of blanching time was modulated by the interactions with the homogenization time and the operation order, whereas for SSC the effect of homogenization time was strongly affected by the operation order (Table 4.2).
In particular, SSC decreased in most of the homogenate products after a short BL-treatment of 80 s, (−5% approx., compared to unblanched purées), but increased with BL-time between 80 s and 240 s (80 s<160 s<240 s) (Table 4.1, Figure 4.5). More extensive heating (320 s) determined a further increase of SSC in HBL purées but a decrease in BLH products (+3.3% and −2.4%, respectively). Also longer H treatments (15 s *vs* 5 s) determined an increase of SSC values in HBL purées but a decrease in BLH products (+3.4%; −2.3%, respectively), between 80 s and 240 s BL-time.

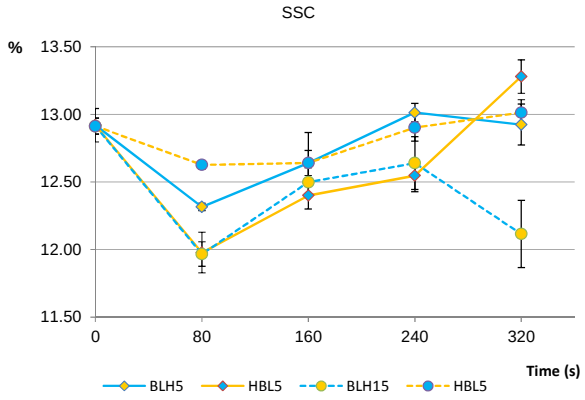

Figure 4.5: Soluble solids (SSC) of blueberry purées as a function of blanching (BL) time (0, 80, 160, 240 and 320 s), homogenization (H) time (5, 15 s) and operation order (H+BL; BL+H). (average of the replicates± std error).

These data could be explained by the combined effect of increased extractability *vs* degradation phenomena operated by mechanical and thermal treatments on fruit constituents. They seem to indicate that these phenomena are governed by the order of BL and H operations, in particular more severe processing conditions (H-time=15 s and BL-time > 240s) enhance solids solubility in HBL products but are detrimental in BLH ones.

Table 4.2: Multifactorial analysis of variance for dry matter, titratable acidity, soluble solids and pH of all purée products.

Source of variation	DM		TA		SSC		pH	
	F-Ratio	P-Value	F-Ratio	P-Value	F-Ratio	P-Value	F-Ratio	P-Value
Main factors:								
A=Blanching time	5,70	***	2,96	*	17,38	***	0,89	0,47
B=Homogenization time	3,08	0,08	2,19	0,14	1,37	0,24	0,49	0,49
C=Operation order	4,07	*	0,36	0,55	4,51	*	7,05	**
Interactions:								
AxB	3,64	**	4,41	**	4,00	**	0,98	0,42
AxC	9,25	***	0,88	0,48	4,83	**	0,86	0,49
BxC	0,57	0,45	4,61	*	19,49	***	0,00	0,97
AxBxC	0,81	0,52	2,27	0,07	1,93	0,11	0,38	0,82

P -value of *F* ratio: **P* <0.05; ***P* <0.01; ****P* <0.001.

[a] blanching time (BL): 0, 80, 160, 240 and 320 s

[b] homogenization time (H): 5 and 15 s

[c] operation order: H+BL; BL+H

Table 4.1: Dry matter (DM, %), titratable acidity (TA, meq NaOH/100g), soluble solids (SSC, %) and pH of blueberry fruits (f) and purée products (p) as a function of blanching (BL) time, homogenization (H) time and operation order (BL+H; H+BL).

	BL_time (s)	H_time (s)										
		0 (f)		5 (p)			15 (p)					
				BL+H		H+BL		BL+H		H+BL		
DM	0	15.65 ± 0.28	a	15.51 ± 0.02	a, A *b*	15.51 ± 0.02	ab, A *b*	15.34 ± 0.04	ab A *a*	15.34 ± 0.04	a, A *a*	***a,a,a***
	80	15.20 ± 0.20	a	15.09 ± 0.15	a, A *a*	15.79 ± 0.15	ab, B *a*	14.87 ± 0.14	a, A *a*	15.59 ± 0.21	a, B *a*	***ab, ab, b, a, ab***
	160	15.53 ± 0.16	a	15.57 ± 0.22	a, A *a*	15.32 ± 0.10	a, A *a*	15.33 ± 0.03	ab A *a*	15.57 ± 0.14	a, A *a*	***a,a,a,a,a***
	240	15.56 ± 0.33	a	15.70 ± 0.18	a, A *a*	15.37 ± 0.12	a, A *a*	15.98 ± 0.19	c, A *a*	15.62 ± 0.09	a, A *a*	***a,a,a,a,a***
	320	15.74 ± 0.14	a	15.73 ± 0.11	a, A *b*	15.97 ± 0.12	b, A *b*	15.39 ± 0.09	b, A *a*	15.56 ± 0.11	a, A *a*	***ab, ab, b, a, ab***
TA	0	12.31 ± 0.44	c	11.95 ± 0.06	ab A *a*	11.95 ± 0.06	a, A *a*	13.89 ± 0.33	b, A *b*	13.89 ± 0.33	b, A *b*	***a, a, b***
	80	9.50 ± 0.31	a	11.49 ± 0.81	a, A *a*	12.42 ± 0.63	a, A *a*	11.25 ± 0.41	a, A *a*	11.50 ± 0.52	a, A *a*	***a, ab, b, ab, ab***
	160	10.90 ± 0.40	b	13.05 ± 0.27	ab A *a*	11.59 ± 0.83	a, A *a*	12.78 ± 0.84	ab A *a*	12.74 ± 0.23	ab, A *a*	***a, a, a, a, a***
	240	11.34 ± 0.15	bc	12.45 ± 0.30	ab A *a*	11.62 ± 0.60	a, A *a*	12.81 ± 0.44	ab A *a*	12.84 ± 0.66	ab, A *a*	***a, a, a, a, a***
	320	11.76 ± 0.32	bc	13.79 ± 0.66	b, B *a*	11.89 ± 0.49	a, A *a*	11.21 ± 0.80	a, A *a*	12.81 ± 0.12	ab, A *a*	***ab, b, ab, a, ab***
SSC	0	12.80 ± 0.13	ab	12.92 ± 0.12	b, A *a*	12.92 ± 0.12	bc, A *a*	12.91 ± 0.06	c, A *a*	12.91 ± 0.06	ab, A *a*	***a, a, a***
	80	12.34 ± 0.11	a	12.32 ± 0.03	a, A *b*	11.98 ± 0.29	a, A *a*	11.97 ± 0.09	a, A *a*	12.63 ± 0.03	a, B *b*	***ab, ab, a, a, b***
	160	12.88 ± 0.21	ab	12.64 ± 0.23	ab A *a*	12.40 ± 0.09	ab, A *a*	12.50 ± 0.11	ab A *a*	12.64 ± 0.09	a, A *a*	***a, a, a, a, a***
	240	12.93 ± 0.34	ab	13.01 ± 0.07	b, B *a*	12.55 ± 0.06	ab, A *a*	12.64 ± 0.20	bc A *a*	12.90 ± 0.10	ab, A *b*	***a, a, a, a, a***
	320	13.20 ± 0.20	b	12.92 ± 0.15	b, A *b*	13.28 ± 0.04	c, B *b*	12.12 ± 0.25	ab A *a*	13.01 ± 0.10	b, B *a*	***b, b, b, a, b***
pH	0	3.15 ± 0.03	a	3.08 ± 0.09	a, A *a*	3.08 ± 0.09	a, A *a*	2.97 ± 0.06	a, A *a*	2.97 ± 0.06	a, A *a*	***a, a, a***
	80	3.23 ± 0.08	a	3.08 ± 0.03	a, A *a*	3.17 ± 0.12	a, A *a*	3.04 ± 0.04	a, A *a*	3.15 ± 0.10	a, A *a*	***a, a, a, a, a***
	160	3.14 ± 0.03	a	3.02 ± 0.04	a, A *a*	3.08 ± 0.06	a, A *a*	2.95 ± 0.06	a, A *a*	3.07 ± 0.05	a, A *a*	***b, ab, ab, a, ab***
	240	3.11 ± 0.03	a	2.99 ± 0.04	a, A *a*	3.00 ± 0.07	a, A *a*	3.03 ± 0.05	a, A *a*	3.13 ± 0.11	a, A *a*	***a, a, a, a, a***
	320	3.15 ± 0.02	a	2.91 ± 0.03	a, A *a*	3.17 ± 0.10	a, B *a*	2.98 ± 0.06	a, A *a*	3.09 ± 0.08	a, A *a*	***a, a, a, a, a***

Values are means ± standard error, n=6

Means follower by different letters are significantly different at P<0.05% (Tukey's test).

(a) small letters in the same column: influence of blanching time within the same product

(A) capital letters within the same row and homogenization time: influence of processing order

(*a*) small letters italics within the same row and processing order: influence of homogenization time

(***a***) small letters italics bold within the same row and blanching time: influence of the product

4.2.2. Influence of processing variables on hydration properties of blueberry purées

Differences among liquid-retaining features of the homogenate products were visible even to the naked eye soon after processing. Syneresis phenomena occurred in some samples within their test tubes, while they were absent in other samples but could be induced by simply removing the matrices from their native position. Hydration properties play a central role both on sensorial, nutritional and textural attributes of the products (Kunzek et al., 1999; Guillon & Champ, 2000; Bayod, 2005; Dhingra et al., 2012; Moelants et al., 2014). Hence various procedures were applied to measure the influence of processing conditions on the strength by which purée products retain their liquid fraction. The amount of liquid lost from the matrices was measured under different and increasing external forces and swelling properties of the homogenate particle components were studied. Average values for hydration parameters of purées are reported in Table 4.3 while results of analysis of variance are shown in Table 4.4.

4.2.2.1. Paper infiltrated area

The liquid-infiltrated area left by a fixed amount of homogenate product laying on a filter paper disk (Figure 4.6) could be considered an indirect measure of the amount of liquid released from the sample itself, under mixed gravity and capillary/suction force (FP; mm^2/g) (Van Oeckel et al., 1999). Higher FP values indicate higher amount of expressed liquid and lower liquid retaining capacity of the samples.

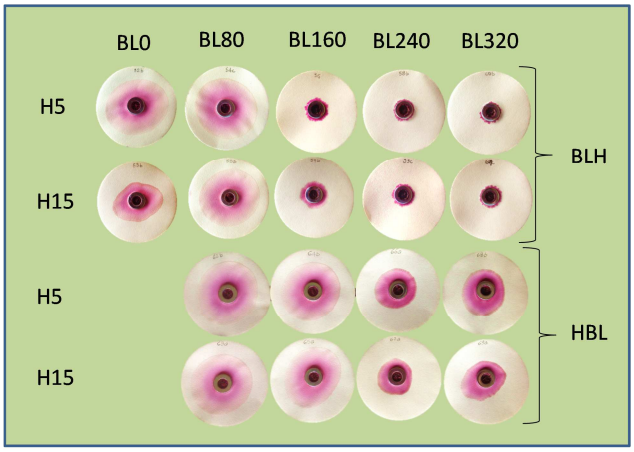

Figure 4.6: Filter-paper test for the study of hydration properties of blueberry purées as a function of blanching (BL) time, homogenization (H) time and operation order.

FP parameter was significantly influenced by all the three main factors with the operation order having the highest impact and the homogenization time the lowest. The effect of blanching time was modulated by the interactions with the homogenization time and the operation order (Table 4.4).

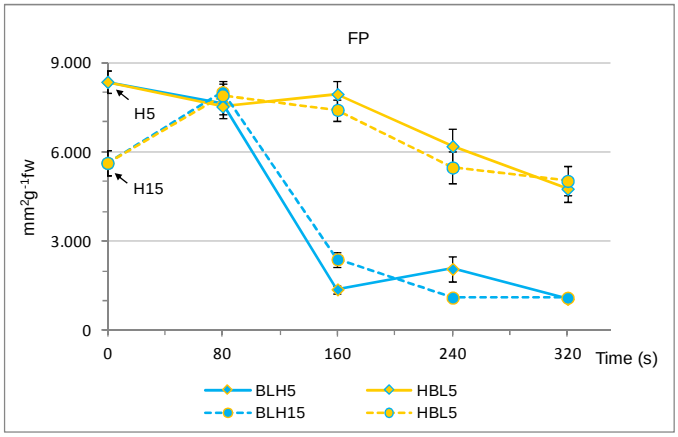

Figure 4.7: Liquid infiltrated area (FP) left by blueberry purées as a function of blanching (BL) time (0, 80, 160, 240 and 320s), homogenization (H) time (5, 15 s) and operation order. (Average of the replicates ± std error).

Considering the FP values of purée products in function of BL-time (Table 4.3, Figure 4.7), in unblanched homogenate products (BL=0), longer H time produced lower FP values (5629 mm^3/g and 8352 mm^3/g for H15 and H5, respectively) but this difference between homogenization times was not found after the mild BL treatment of 80 s. However, as BL time increased, FP values decreased and at higher rate and to a higher extent in BLH samples than in HBL purées, whatever the H time. In particular, the 160 s BL treatment produced a marked decrease (−70%) of FP values in BLH samples but had little or no effect in HBL products. Longer BL treatments were necessary to produce a little decrease of FP values in HBL products (−27% after 320s BL-time) while they had little further effect in BLH samples. As a result, the average liquid infiltrated area of thermally treated purées in HBL samples was three times as much as BLH samples. We can conclude that liquid retaining feature of purées measured by the FP method decreased with H time in unblanched product while it increased with BL time in thermally treated products but this increase occurred faster and it was much more pronounced if the BL treatments were preliminary operated on whole fruits (BLH purées) than on already homogenate matrices (HBL purées).

4.2.2.2. Expressible liquids

The amounts of liquid released from purée products under natural gravity at two weight-forces ($G1_{0.5g}$ and $G2_{20g}$) and under centrifugal enhanced gravity (C1) were registered and expressed as percentage of the initial samples weight. In Figure 4.8, average values of G1, G2 and C1 expressible liquids are reported for all the purée products as a function of BL-time. Whatever the procedure applied to measure it, the amount of liquid released from the samples was strongly influenced by the operation order, and then by BL time, while H time had a significant influence only on C1 parameter. The effects of the operation order were modulated by the interaction with the BL time for all the three parameters, by the interaction with H time for G2 and by the interaction with BL time and H time for G1 and G2. Moreover, for all the three parameters the effects of BL time were modulated by the interaction with H time (Table 4.4).

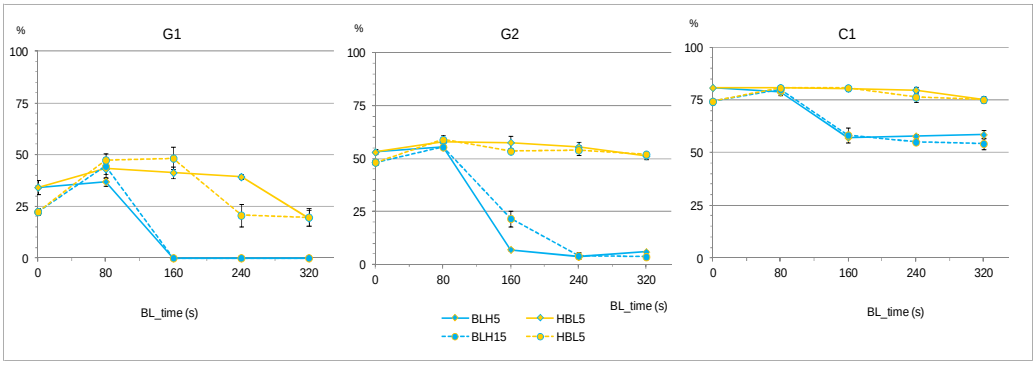

Figure 4.8: G1, G2 and C expressible liquids (%) of blueberry purées as a function of blanching (BL) time (0, 80, 160, 240 and 320s), homogenization (H) time (5, 15 s) and operation order (average of the replicates ± std error).

In unblanched purées (BL=0s), the overage amount of liquid released from the matrices against low, medium and enhanced gravity was respectively 28.2% (G1), 50.7% (G2) and 77.5% (C1) of the initial sample weight and, within each parameter, higher H-time produced lower expressible liquids values (H15 *vs* H5).

After a short BL-treatment (80s) a small increase of the expressible liquid amount was registered (+12.4%, +4.8% and +1.7% for G1, G2 and C1, respectively) and a tendency of BLH samples to retain higher amount of liquids compared to HBL ones was evident for G1 (low external force).

Longer BL treatments produced a decrease in expressible liquid amount, but this decrease occurred earlier and it was much more pronounced in BLH purées compared to HBL ones, underlying a strong influence of the operation order on liquid holding properties of the matrices. In particular, at BL$\geq$160s, syneresis phenomena were totally prevented (G1) or largely reduced (G2, C1) in BLH purées, while they were reduced to a less extent (G1) or unaffected (G2, C1) in HBL products.

Furthermore, increasing the external force applied (G1<G2<C1), data trend among samples was maintained but the average amount of liquid released from purées increased, even if differences among samples decreased. As a result, at BL=320s, the average amount of liquid released against low, medium and enhanced gravity was respectively 0% (G1), 5% (G2) and 55% (C1) of the initial sample weight for BLH matrices and 20% (G1), 50% and 75% (C1) for HBL purées.

The results on expressible liquids suggested that with the increase of the external force applied, more tightly bound liquid fractions could be separated from the products. Hence, differences among loosely-bound liquid fractions of the samples could be detected only against low external force (G1). On the contrary, at enhanced centrifugal force (C1), differences among samples could be detected with respect to more tightly bound or entrapped liquid fractions.

In particular, if we subtract G2 to C1 percentage values (Fig. 4.9), we observe that enhanced gravity force produced higher increases in the expressible liquid amount for BLH purées, suggesting that in these products, compared to HBL ones, higher amounts of entrapped or loosely bound water were present, which needed a higher external force to be released.

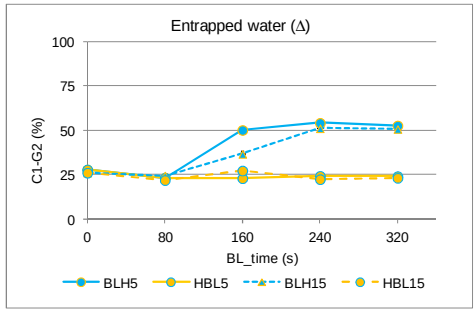

Figure 4.9: Delta between G2 and C1 expressible liquids (%) of blueberry purées as a function of blanching (BL) time, homogenization (H) time and operation order.

Hence, the operation order might act on tightly-bound water, whereas the blanching time on the loosely-bound water .

4.2.2.3. Swelling capacity

For all purées, settled volume of particle components at maximum hydration was measured (swelling capacity, SC). In Figure 4.10, average SC values of purées are reported as a function of BL-time.
Multifactorial analysis of variance indicates that the main factors affecting SC were BL time and operation order with this last having the highest impact on the parameter. Besides, the influence of BL-time was strongly modulated by the interaction with operation order and to a less extent with H time (Table 4.4).
In unblanched purées (BL=0 s) the higher H-time produced higher SC values (3170 mm^3g^{-1} and 2320 mm^3g^{-1} for H15 and H5, respectively). Short BL-treatments (80 s) produced a little decrease of the settled volume of purées, whatever the operation order (−30% approx). In contrast, for BL>80 s a different swelling behaviour was found in function of the operation order. In BLH samples the swelling capacity of purées dramatically increased with BL-time (+90%, +139%, +159% at 160 s, 240 s and 320s BL-time, respectively), while this increase was very low in HBL samples (+8%, +20%, +36% at 160 s, 240 s and 320 s BL-time, respectively). Furthermore, within the same operation order, the higher H-time produced a faster SC increase (H15 vs H5).

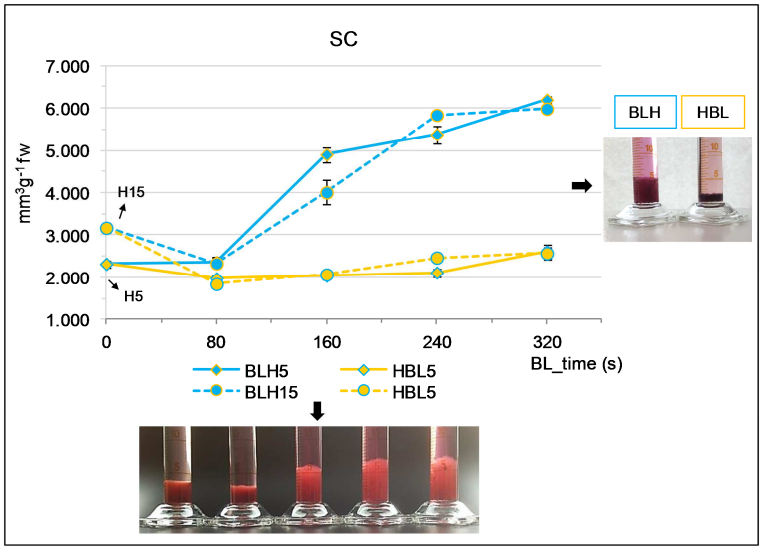

Figure 4.10: Swelling capacity (SC, mm^3g^{-1}) of blueberry purées as a function of blanching (BL) time (0, 80, 160, 240 and 320 s), homogenization (H) time (5, 15 s) and operation order. (Average of the replicates ± std error).

The swelling capacity of a plant food is a measure of the volume occupied in a liquid medium by its particles, also including their sphere of interaction with surrounding components (dynamic volume). This parameter is strictly related to hydration properties of dispersed cell wall materials (CWM) such as cellulose, hemicellulose, lignin and pectins (Dhingra et al., 2012). The swelling capacity of a fruit pulp is a parameter physiologically relevant because it has a role in determining gastric fill, transit rate and satiety (Takahashi et al., 2009). Heat-treatments may affect cross-linking between cell wall constituents, decreasing packing of fibers and increasing their solubility. Besides, homogenization reduces particles size increasing their surface area.
In blueberry purées, a preliminary fruit blanching step (BLH products) produced a significant increase in the swelling capacity of the derived homogenates, settled particles reaching almost three times their initial volume after a BL treatment of 320 s. However, the blanching step carried out on already homogenate berries (HBL products) was much less effective towards SC.
We can hypothesize that the effect of mechanical forces on dynamic volume of CWM was much more pronounced in BLH samples because fruit fibers had been preliminary loosened by heat treatments (BL≥80s). On the contrary, in HBL purées, the preliminary H-treatments increase the surface area of CWM but not their dynamic volume (SC).

4.2.2.4. Water activity

Water activity (a_w) is a thermodynamic measure of the energy of water in a product. Both solutes concentration (osmotic effects) and surface phenomena (matrix effect) may affect this parameter (Maltini et al., 2003). In foods, a_w is related to microbial susceptibility and degradative reactions.

Our samples were characterized by a_w values between 0.985 and 0.987, which were in the range of a_w values reported for fruit and vegetable juices (Gabriel et al., 2008). A limited influence of BL-time on a_w of purées was registered, probably related to heat-induced solubilisation phenomena, while H-time and operation order did not affect this parameter (Table 4.4).

Table 4.4: Multifactorial analysis of variance for hydration parameters of blueberry purées: paper infiltrated area FP (mm^2/g fw), expressible liquid fractions G1 (%), G2 (%) and C1 (%), swelling capacity SC (mm^3/g fw) and water activity (aw).

Source of variation	FP		G1		G2		C1		SC		aw	
	F-Ratio	P-Value	F-Ratio	P-Value	F-Ratio	P-Value	F-Ratio	P-Value	F-Ratio	P-Value	F-Ratio	P-Value
Main factors:												
A=Blanching time	119,47	***	74,16	***	248,08	***	75,19	***	199,74	***	3,50	**
B=Homogenization time	10,94	**	3,03	0,08	0,01	0,91	10,44	**	4,78	*	0,68	0,41
C=Operation order	264,42	***	217,59	***	1472,70	***	383,59	***	1168,92	***	1,68	0,20
Interactions:												
AxB	11,86	***	6,86	***	4,75	**	4,08	**	16,08	***	2,11	0,08
AxC	49,54	***	37,69	***	226,21	***	58,00	***	165,26	***	1,66	0,16
BxC	0,39	0,53	1,15	0,29	4,84	*	0,04	0,85	2,51	0,11	2,16	0,14
AxBxC	0,98	0,42	2,55	*	7,17	***	0,55	0,70	2,94	*	1,01	0,40

P -value of *F* ratio: **P* <0.05; ***P* <0.01; ****P* <0.001.

[a] blanching time (BL): 0, 80, 160, 240 and 320 s

[b] homogenization time (H): 5 and 15 s

[c] operation order: H+BL; BL+H

Table 4.3: Average values for expressible liquid fractions of blueberry purées evaluated by filter-paper method (FP, mm^2g^{-1}), filter-washer method (G1, %), polyester−mesh method (G2, %) and centrifuge method (C1, %), as far as average values for swelling capacity (SC, mm3g-1) and water activity (aw) of the products, reported as a function of blanching (BL) time, homogenization (H) time and operation order (BL+H; H+BL).

	BL_time (s)	H_time (s)												
		5						15						
		BL+H			H+BL			BL+H			H+BL			
FP	0	8352 ± 385	b	A *b*	8352 ± 385	c	A *b*	5630 ± 419	c	A *a*	5630 ± 419	ab	A *a*	***b, a***
	80	7628 ± 359	b	A *a*	7527 ± 406	bc	A *a*	8007 ± 275	d	A *a*	7900 ± 466	c	A *a*	***a, a, a, a***
	160	1382 ± 124	a	A *a*	7940 ± 418	bc	B *a*	2391 ± 238	b	A *b*	7413 ± 358	bc	B *a*	***a, b, a, b,***
	240	2069 ± 442	a	A *b*	6193 ± 561	ab	B *a*	1103 ± 69	a	A *a*	5475 ± 549	a	B *a*	***a, b, a, b***
	320	1064 ± 70	a	A *a*	4758 ± 422	a	B *a*	1099 ± 40	a	A *a*	5027 ± 497	a	B *a*	***a, b, a, b***
G1	0	34.20 ± 3.44	b	A *b*	34.20 ± 3.44	b	A *b*	22.35 ± 1.54	b	A *a*	22.35 ± 1.54	b	A *a*	***b, a***
	80	36.98 ± 2.07	b	A *a*	43.13 ± 4.12	b	A *a*	44.36 ± 4.04	c	A *a*	47.34 ± 3.30	b	A *a*	***a, a, a, a***
	160	0.00 ± 0.00	a	A *a*	41.28 ± 2.78	b	B *a*	0.00 ± 0.00	a	A *a*	48.26 ± 5.55	a	B *a*	***a, b, a, b***
	240	0.00 ± 0.00	a	A *a*	39.28 ± 1.35	b	B *b*	0.00 ± 0.00	a	A *a*	20.64 ± 5.43	a	B *a*	***a, b, a, b***
	320	0.00 ± 0.00	a	A *a*	19.22 ± 3.85	a	B *a*	0.00 ± 0.00	a	A *a*	19.68 ± 4.20	a	B *a*	***a, b, a, b***
G2	0	53.07 ± 1.16	b	A *a*	53.07 ± 1.16	a	A *a*	48.26 ± 1.52	c	A *a*	48.26 ± 1.52	a	A *a*	***a, a***
	80	55.47 ± 0.66	b	A *a*	57.71 ± 0.81	a	A *a*	55.45 ± 0.62	c	A *a*	58.88 ± 2.01	b	A *a*	***a, a, a, a***
	160	7.10 ± 0.51	a	A *a*	57.62 ± 2.92	a	B *a*	21.69 ± 3.84	b	A *b*	53.47 ± 0.40	ab	B *a*	***a, c, b, c***
	240	3.79 ± 0.57	a	A *a*	55.52 ± 2.34	a	B *a*	4.06 ± 1.61	a	A *a*	54.03 ± 2.28	ab	B *a*	***a, b, a, b***
	320	6.02 ± 0.42	a	A *a*	51.26 ± 1.74	a	B *a*	3.69 ± 0.84	a	A *a*	52.00 ± 0.64	ab	B *a*	***a, b, a, b***
C1	0	80.82 ± 0.16	b	A *b*	80.82 ± 0.16	a	A *b*	74.25 ± 0.66	b	A *a*	74.25 ± 0.66	a	A *a*	***b, a***
	80	78.65 ± 1.51	b	A *a*	80.77 ± 1.30	a	A *a*	79.86 ± 0.48	b	A *a*	80.69 ± 0.13	b	A *a*	***a, a, a, a***
	160	57.11 ± 0.44	a	A *a*	80.49 ± 1.41	a	B *a*	58.26 ± 3.47	a	A *a*	80.65 ± 0.47	b	B *a*	***a, b, a, b***
	240	57.80 ± 0.76	a	A *a*	79.68 ± 1.29	a	B *a*	55.01 ± 1.23	a	A *a*	76.35 ± 2.29	ab	B *a*	***a, b, a, b***
	320	58.55 ± 2.05	a	A *a*	75.28 ± 1.64	a	B *a*	54.25 ± 2.67	a	A *a*	75.07 ± 1.05	a	B *a*	***a, b, a, b***
SC	0	2320 ± 83	a	A *a*	2320 ± 83	ab	A *a*	3172 ± 114	b	A *b*	3172 ± 114	c	A *b*	***a, b***
	80	2365 ± 133	a	B *a*	1976 ± 55	a	A *a*	2332 ± 109	a	B *a*	1839 ± 74	a	A *a*	***c, ab, bc, a***
	160	4900 ± 177	b	B *b*	2036 ± 76	a	A *a*	4005 ± 289	c	B *a*	2068 ± 45	a	A *a*	***c, a, b, a***
	240	5377 ± 203	b	B *a*	2112 ± 100	a	A *a*	5837 ± 63	d	B *b*	2469 ± 99	b	A *b*	***b, a, b, a***
	320	6198 ± 70	c	B *b*	2612 ± 155	b	A *a*	5984 ± 69	d	B *a*	2573 ± 140	b	A *a*	***b, a, b, a***
aw	0	0.9850 ± 0.0005	a	A *a*	0.9850 ± 0.0005	a	A *a*	0.9856 ± 0.0004	a	A *a*	0.9856 ± 0.0004	a	A *a*	***a, a, a***
	80	0.9861 ± 0.0004	a	A *a*	0.9863 ± 0.0003	ab	A *a*	0.9867 ± 0.0003	a	A *a*	0.9862 ± 0.0002	a	A *a*	***a, a, a, a, a***
	160	0.9860 ± 0.0006	a	A *a*	0.9868 ± 0.0002	b	A *b*	0.9855 ± 0.0004	a	A *a*	0.9856 ± 0.0004	a	A *a*	***a, a, a, a, a***
	240	0.9848 ± 0.0005	a	A *a*	0.9867 ± 0.0003	ab	B *a*	0.9859 ± 0.0003	a	A *a*	0.9860 ± 0.0003	a	A *a*	***ab, a, b, ab, ab***
	320	0.9855 ± 0.0004	a	A *a*	0.9852 ± 0.0006	ab	A *a*	0.9860 ± 0.0003	a	A *a*	0.9861 ± 0.0004	a	A *a*	***a, ab, ab, b,b***

Values are means ± standard error, n=6

Means follower by different letters are significantly different at P<0.05% (Tukey's test).

(a) small letters in the same column: influence of blanching time within the same product

(A) capital letters within the same row and homogenization time: influence of processing order

(*a*) small letters italics within the same row and processing order: influence of homogenization time

(***a***) small letters italics bold within the same row and blanching time: influence of the product

4.3. Conclusions

Multifactorial analysis of variance performed on all the homogenate products indicate that all the hydration measurements considered were deeply affected by the process, a_w to a less extent. Blanching time and operation order significantly influenced FP, G1, G2, C1 and SC parameters, while homogenization time exhibited an influence on FP and C1 values but not on G1, G2 and SC. Significant interactions between blanching time and homogenization time and blanching time and operation order were found for all the parameters. The liquid retaining feature of purées increased with BL time; BLH purées had higher amounts of entrapped or loosely bound water than HBL ones, indicating that operation order might act on tightly-bound water, whereas the blanching time on the loosely-bound water. The effect of mechanical forces on dynamic volume of CWM was much more pronounced in BLH samples because fruit fibers had been preliminary loosened by heat treatments, whereas in HBL purées the preliminary H-treatments increased the surface area of CWM but not their dynamic volume (SC).

4.4. References

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5. TOPIC 3

Phenolic composition of the homogenate particle−liquid system

Phenolic constituents are partitioned all among the particle and liquid components of the homogenate system, and in function of this distribution they exhibit different mobility behaviours and degree of accessibility. Higher mobility was attributed to the phenolic component dissolved into the homogenate liquid fraction, which can be easily released from the processed matrices, while lower mobility was attributed to molecules bound or entrapped into the homogenate particle fraction.

On these bases, in this section it was studied the influence of processing on anthocyanin and phenolic composition of blueberry fruits and homogenate matrices, distinguishing between dissolved and particle−associated phenolic molecules. Dissolved phenolic compounds were collected within the liquid fraction separated under natural (G2) or enhanced (C1) gravity from the products, while polyphenols associated to the tissue particles were extracted by selected solvent (C2, C3). Liquid fractions and extracts were analyzed for total phenolic compounds, colour indices as far as for total and individual anthocyanin compounds by applying spectrophotometric and chromatographic techniques. Extraction of compounds from whole homogenate and fruit samples (E) was also carried out.

5.1. Material and methods

5.1.1. Experimental set up

Homogenate systems were separated into fractions of decreasing mobility and phenolic composition of the separated fractions was assessed. Figure 5.1 shows the flow−chart for the preparation of phenolic fractions from blueberry homogenates. Maximum accessibility was attributed to phenolic compounds conveyed by the homogenate liquid fraction free to move under gravity (gravitational liquid fraction, G2), followed by: i) phenolics associated to the homogenate loosely bound or entrapped liquid fraction, released under centrifugal force (centrifugal liquid fraction, C1); ii) loosely associated phenolics, released from homogenate particles by diluted formic acid (C2), and iii) tightly bound phenolics, extracted by acidified acetone (C3). Hence the homogenate polyphenols were separated into liquid and particle phases ones, and particle-bound components were extracted by the selected solvents of increasing strength. Extraction of compounds from whole homogenate and fruit samples was also carried out. Liquid fractions and extracts were analyzed for total phenolic compounds, monomeric anthocyanin pigments and colour indices by applying spectrophotometric measurements as far as for individual anthocyanin compounds and chlorogenic acid by chromatographic technique.

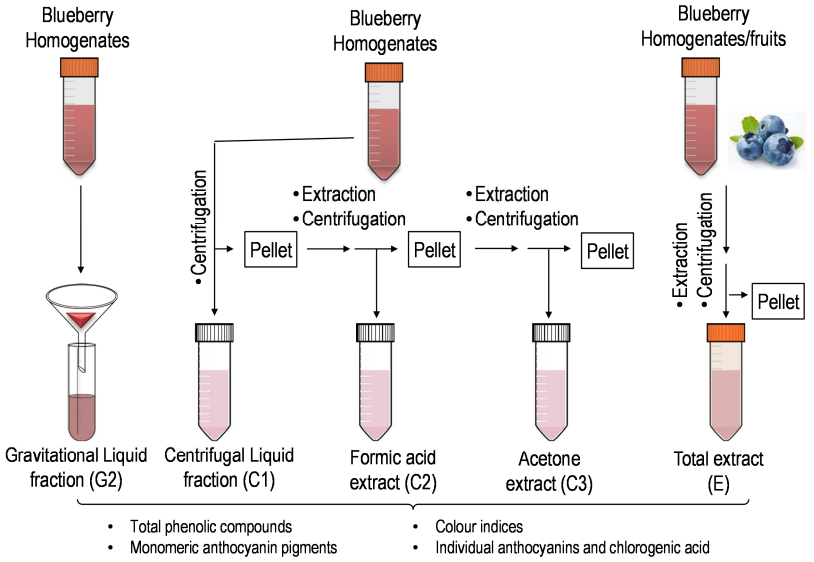


Figure 5.1: Flow-chart of samples for the study of phenolic accessibility

5.1.2. Phenolic liquid fractions

5.1.2.1. Gravitational liquid fraction (G2)

Free or gravitational liquid fraction (G2) was separated from homogenate samples according to the procedure reported in section 4.1.1.2.

5.1.2.2. Centrifugal liquid fraction (C1)

The sum of free and entrapped or loosely-bound liquid fractions (C1) was obtained from homogenate samples under centrifugal force, according to the procedure reported in section 4.1.1.4.

5.1.3. Phenolic particle-bound fractions

5.1.3.1. Formic acid extract (C2)

Pellets remaining after the removal of the C1 supernatants were broken up with a spatula and then were extracted by stirring for 60 min in ice-water bath covered with an aluminum foil with an amount of formic acid (1% v/v) equal to the amount the removed supernatant. After centrifuging at 16,000 × *g* for 20 min at 4 °C the supernatant (C2) was weighted and stored at −20 °C till phenolic analysis, while pellets were submitted to further extraction according to the procedure described in section 5.1.3.2..

5.1.3.2. Acetone extract (C3)

Pellets remaining after the removal of the C2 supernatants were broken up and re-extracted by adding 30 ml of a solvent containing water, acetone and formic acid (79:20:1 v/v/v). The acetone solution was chosen on the basis of previous investigations which report for this solvent a good extraction rate of anthocyanins from the skin of fruits (Brambilla et al, 2008; Dai et al, 2010; Koddami et al., 2013). After 48 h at 4 °C in the dark, the homogenates were stirred for 60 min in ice-water bath and centrifuged at 16,000 × *g* for 20 min at 4 °C. The supernatant (C2) was weighted and stored at −20°C till phenolic analysis, while pellets were discharged.

5.1.4. Total phenolic extract (E)

Total phenolics were extracted from the whole fruit and the whole homogenate samples. For the extraction a solvent was employed containing water, acetone and formic acid (79:20:1 v/v/v) and a maceration procedure was developed which includes a single extraction step, with a final sample-to-solvent ratio of 1:10 (w/w) and operations being carried out at controlled temperature (4 °C) in the dark. Maceration procedures are usually applied to enable the release of polyphenols from skin and seed compartments of grape berries (Catalano et al., 2013). Frozen samples (15 g) were immersed in an aliquot of the extracting medium (55 g), kept for 1 h in a thermostatic chamber at 4 °C and homogenized for 60 s using an Ultraturrax blender (IKA Labortechnik, Germany) at low speed. After 48 h at 4°C in the dark, the homogenates were further diluted to 150 g with the extracting medium, stirred for 30 min in ice-water bath covered with an aluminum foil and centrifuged at 4,000 × *g* for 30 min at 4° C. Both the supernatant (E = total phenolic extract) and the pellet were collected and stored at −20°C until analysis.

5.1.5. Spectrophotometry

Liquid fractions (G2 and C1) and extracts (C2, C3 and E) were analyzed in duplicate for total phenolic compounds, monomeric anthocyanin pigments and colour indexes using a Jasco 7800 UV/Vis spectrophotometer (Jasco, Easton, MD). For all determinations G2 and C1 liquid fractions were preliminary diluted by 10 with formic acid (1% v/v) while C2, C3 and E extracts were analyzed undiluted.

5.1.5.1. Total phenolic compounds

Total phenolic compounds (TPC) were determined by Folin-Ciocalteau micro method, as reported by Waterhouse (2001). Calibration curves for post-run quantification were prepared daily with standard phenols concentrations of 0, 50, 100, 250 and 500 mg/L gallic acid (Sigma-Aldrich) and phenolic content of the samples was expressed as milligrams of equivalent gallic acid per 100 g of sample (mg GAE/100g).

5.1.5.2. Monomeric anthocyanin pigments

Monomeric anthocyanin pigments (MAP) were estimated by pH-differential method according to Giusti & Wrolstad (2001). Briefly, sample extracts were diluted in two buffer solutions at pH 1 (anthocyanins in the coloured oxonium or flavilium form) and pH 4.5 (anthocyanin in the colourless carbinol form) and MAP content was calculated according to Eq. 5.1:

$$\text{MAP (mg L}^{-1}) = \frac{A \times MW \times DF \times 1000}{\varepsilon \times l} \qquad (5.1)$$

where: A is the absorbance computed according to Eq. 5.2:

$$A = (A_{\lambda\text{vis-max}} - A_{700\text{nm}})_{\text{pH1.0}} - (A_{\lambda\text{vis-max}} - A_{700\text{nm}})_{\text{pH4.5}} \qquad (5.2)$$

MW is cyanidin-3-glucoside (C3G) molar mass (449.2 g mol^{-1}), DF the dilution factor, ε the C3G molar absorption coefficient (26,900 L cm^{-1}mol^{-1}) and *l* the path length. Results were expressed as milligrams of equivalent cyanidin-3-glucoside per 100 g of sample (mg C3GE/100 g).

5.1.5.3. Colour Indices

Colour Indices were measured by the bisulfite bleaching method developed by Somers (1971), following the procedure described by Giusti & Wrolstad (2001). This method is based on the ability of bisulfite to form colourless adducts with monomeric anthocyanin compounds but not with polymeric pigments. Sample extracts were diluted with water (control samples, Cs) or with a potassium metabisulfite solution (bleached samples, Bs) and the absorbances of both dilutions were evaluated at 700 nm (haze), 512 nm (anthocyanin), and 420 nm (brown pigments). Colour density (CD), polymeric colour (PC), percent polymeric colour (%PC) and browning index (BI) were calculated according to Eq. 5.3 – 5.6:

$$\text{CD} = [(A_{420\text{nm}} - A_{700\text{nm}}) + (A_{512\text{nm}} - A_{700\text{nm}})]_{\text{Cs}} \times \text{DF} \qquad (5.3)$$

$$\text{PC} = [(A_{420\text{nm}} - A_{700\text{nm}}) + (A_{512\text{nm}} - A_{700\text{nm}})]_{\text{Bs}} \times \text{DF} \qquad (5.4)$$

$$\%\text{PC} = \frac{PC}{CD} \times 100 \qquad (5.5)$$

$$\text{BI} = (A_{420\text{nm}})_{\text{Bs}} \qquad (5.6)$$

5.1.6. High Performance Liquid Chromatography

Due to the duration of each single analysis, the number of samples forseen by the experimental plan shown in Table 3.1 and the number of extracts obtained from each sample as summarized in Figure 5.1, the choromatographic analyses were performed on extracts from samples of the third day replicate of processing.

5.1.6.1. Individual anthocyanins and chlorogenic acid

Sample ertracts were analyzed in duplicate by gradient-RP-HPLC and diode array detection for chlorogenic acid content (mg CA/100g fw) and individual anthocyanin (ACN) content, expressed as cyanidin 3-glucoside equivalents (mg C3GE/100g fw). The chromatographic system consisted of a PU 1580 pump (Jasco Co., Tokyo, Japan), a 250 × 4.6 mm I.D., 5 µm, Kinetex EVO column fitted with a SecurityGuard Ultra cartridge (Phenomenex, USA), kept at 40°C and a Jasco MD 2010 Plus photodiode array detector. The mobile phases consisted of acetonitrile (A) and water−formic acid (95:5, v/v) (B) and elution was performed at a flow rate of 0.5 mL/min by linear gradient steps (Brambilla et al., 2008). Before injection (20 µl) the extracts were diluted 1:10 (v/v) with the mobile-phase B and filtered through a 0.45 µm nylon filter unit (Chromacol, United Kingdom). Concentrations of individual ACNs (520 nm) and CA (320 nm) were calculated using an external calibration curve of the C3G (Extrasynthese) and CA (Sigma-Aldrich) standard compounds.

5.1.7. Statistical analysis

Statistical analysis was performed using Statgraphics version 7 (Manugistic Inc, Graphics Software Systems, Rockville, MD, USA) software. Data were processed by multifactor analysis of variance (ANOVA) considering blanching (BL) time, homogenization (H) time, operation order and their interactions as source of variation. Furthermore one-way ANOVA was used to study: (a) the influence of BL-time within the same product, (b) the influence of processing order within the same H-time, (c) the influence of H-time within the same processing order, (d) the influence of the product. Means were compared by Tukey's test at $P \leq 0.05$.

5.2. Results and discussion

5.2.1. Spectrophotometric measurements

The results of the analysis of variance for total phenolic compounds (TPC), monomeric anthocyanin pigments (MAP) and colour indices of the phenolic fractions are reported in Table 5.1, while the average values for all parameters are reported in. Tables 5.2 - 5.6.

5.2.1.1 Total phenolic extract (E)

The total phenolic extract of fruit and homogenate samples was analyzed in order to assess the influence of processing variables on the overall phenolic profile of purées.
To this aim, total phenolics were recovered from the matrices by applying a maceration procedure involving a single extraction step (E-extract).
Average TPC and MAP values of the E-extracts of berries and purées in function of BL-time are reported in Figure 5.2.

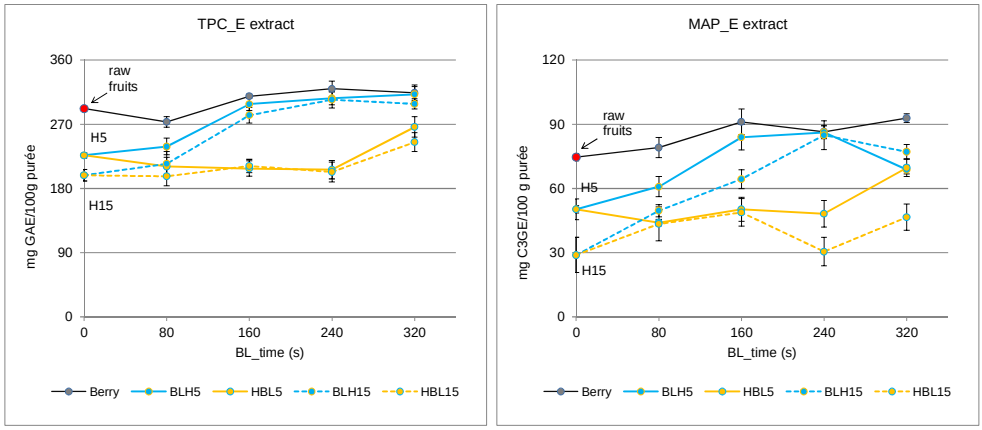


Figure 5.2: Total phenolic compounds (TPC, mg GAE/100g) and monomeric anthocyanin pigments (MAP, mg C3GE/100 g) of the E-extract of blueberry fruit and purée products as a function of blanching (BL) time, homogenization (H) time and operation order (BL+H; H+BL)**.** (Average of the replicates ± std error).

Blueberry fruit were characterized by a TPC of 292 mg GAE/100g fw, and a MAP of 74.6 mg C3GE/100g fw (Figure 5.2). These values are in agreement with literature data for this blueberry variety (Rodriguez-Mateos et al, 2012).

Blanching induced slight variations in TPC (±8%) and an increase in MAP content of berries (+24%, BL=320 s). Besides, homogenization had a detrimental impact on both parameters. TPC values in fact decreased in raw berries with H-time (−22% and −32% in H5 and H15 samples, respectively) and approx. one-third (−32%) of the MAP was lost after the shorter H-treatment (H=5s), and further decreased (−61%) in extensively blended berries (H=15s).

In purée products, operation order was the main factor affecting both TPC and MAP parameters while H-time had a slight impact on monomeric anthocyanin pigments and BL-time on total phenolic compounds (Table 5.1). Both for TPC and MAP, the influence of the operation order was modulated by the interaction with BL-time.
In BLH purées, TPC content increased with BL-time (+6, +36, +43% at 80 s, 160 s and 240 s BL-time, respectively) and MAP almost triplicated within the same BL-time interval, with the higher H-time producing lower MAP values. On the other hand, the blanching step carried out on already homogenate berries (HBL products) was much less effective towards these parameters. As a result, within the same BL-time, BLH purées had always higher MAP and TPC content compared to HBL purées.
Losses of MAP and TPC coincided with increases of %PC with was significantly higher in HBL products (14%, average value) and BLH products (12%, average value) compared to fruits (8%, average value) (Table 5.2).
These data indicate that:

- In the intact fruit system, where enzymes and substrates are spatially separated, blueberry phenolic and anthocyanin molecules were relatively heat-stable (BL-treatments did not produce a decrease of TPC and MAP in blueberry fruit, unless TPC in BL80). Similar results were also reported by Brownmiller et al. (2008), who analyzed the MAP content of blueberries after a steam blanching treatment of 180s.
- BL-treatments could enhance the release of polyphenols from their fruit native compartments and/or protect them from oxidative degradations during subsequent processing/extraction

procedures (with BL $\geq$ 160s, TPC and MAP content of the E-extract of berries increased), confirming data reported in previous investigations (Brambilla et al., 2011).

- Main losses of phenolic and anthocyanin compounds were associated to the initial homogenization step on raw berries (H5 and H15) and could be ascribed to enzymatic oxidations occurring when oxidative enzymes and phenolic substrates interact in the blended tissues. MAP were more susceptible to mechanical losses than TPC (Patras et al., 2010; Howard et al, 2012).
- Initial losses of phenolics due to mechanical treatments cannot be compensated by a successive BL-treatment (HBL purées); the initial phenolics losses can only be prevented by a preliminary BL-step. The increase of the TPC and MAP values of HBL purées for BL$\geq$240s could be attributed to heat-induced increased extractability phenomena.
- Among purées, the highest TPC and MAP contents were achieved in BLH products, with a BL-time of 240 s, independently of H-time, with $BL_{240}H$ purées showing on average a MAP content of 85.5 mg C3GE/100 g and a TPC ones of 305.4 mg GAE/100 g, values not different from those found for berries. In contrast, at the same BL-time (240 s) in HBL purée half amount of MAP and one-thirds of TPC were lost.

5.2.1.2. Phenolics in G2 and C1 liquid fractions

Easily accessible phenolic compounds were analyzed in the liquid fractions of purèe products. Figure 5.3 compares data of TPC, MAP and %PC of syneresis liquid from purée under natural gravity at the higher weight force (G2) and those of liquid obtained under centrifugal enhanced gravity (C1).

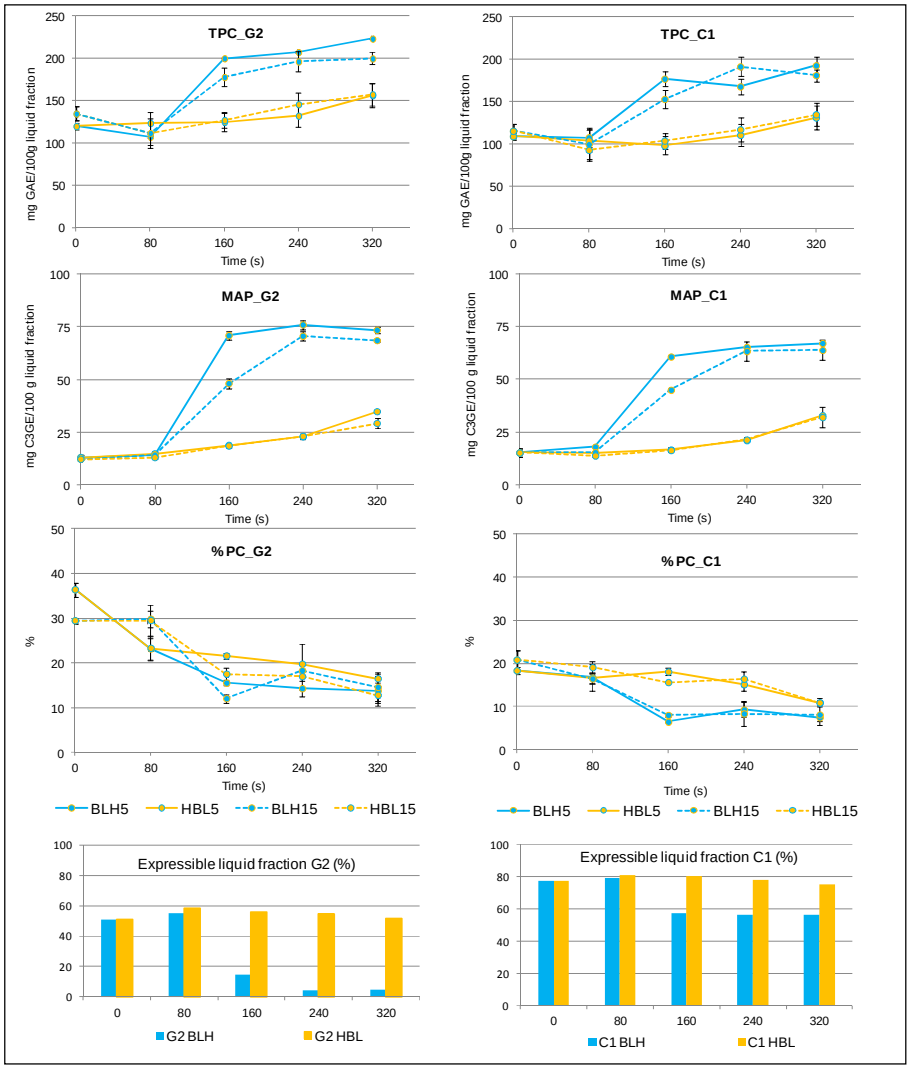


Figure 5.3: Total phenolic compounds (TPC, mg GAE/100 g), monomeric anthocyanin pigments (MAP, mg C3GE/100 g), percent polymeric colour (%PC) and expressible liquid fraction (%) of the G2 and C1 liquid fractions of blueberry purées as a function of blanching (BL) time, homogenization (H) time and operation order (BL+H; H+BL)**.** (Average of the replicates ± std error).

Multifactorial analysis of variance indicates that in both G2 and C1 liquid fractions, TPC content was mainly influenced by operation order and secondly by BL-time, while H-time had no significant effect. MAP content depended on all the main factors and interactions, with operation order and BL-time having the highest impact. In G2, %PC was influenced by BL-time and its effect was modulated by H-time while in C1 %PC was influenced by operation order and BL-time (Table 5.1).

If the average TPC and MAP values referred to 100 g of G2 and C1 liquid (figure 5.3) are considered, in BLH purées they did not vary from 0 to 80 s BL-time, then they steeply increased for BL-time ≥160 s, without any significant further change for BL≥240s. In G2 this increase was higher, while in C1 it happened earlier (160 s *vs* 240 s BL-time).
In contrast, in HBL purées there was only a slight increase of TPC and MAP with BL-time in G2 and C1 liquids, without any influence of H-time.
In G2, %PC decreased as the BL-time increased, with H5 purées showing the lowest values (Table 5.3). In C1 %PC decreased as the BL-time increased, more in BLH than in HBL samples, with BLH C1 liquids showing %PC values lower than 10% for BL-times≥ 160 s (Table 5.4).

As a result, for BL-time>160 s, TPC and MAP were much more concentrated in the G2 and C1 liquid fractions of BLH purées than of HBL pureés and this effect was more pronounced for MAP (+175% and +160% in G2 and C1, respectively) compared to TPC (+43% and +53%, in G2 and C1 respectively).

On the other hand, if the TPC and MAP contents are referred to 100 g of purée instead of to 100 mL of liquid fraction, a different scenario in function of the operation order and of the liquid fraction is found.

In fact, the G2 liquid volume (Figure 5.4, bottom panel) was strongly reduced for BL-times ≥ 160 s when BL-H operation order was applied. As a consequence, for BL-H samples, the high concentrations of TPC and MAP found in the very low volumes of G2 liquid corresponded to approx. 5% of TPC and 5-10% of MAP found in the total extracts from purées. On the other hand, for H-BL samples, the lower concentration of TPC and MAP found in high volumes of G2 liquid corresponded to approx. 35% of TPC and 15-25% of MAP quantified in the total extracts from purées.

Hence, with reference to the gravitational liquid fraction G2, even though the MAP and TPC liquid concentrations were much higher in BLH purées, the rate of these compounds released from the matrices was higher in HBL purées.

Also C1 liquid volume of purées was reduced by a preliminary BL step (BL-times≥ 160 s) but this decrease was less pronounced than for G2 (Figure 5.4, bottom panel).
As a consequence, in C1 liquids, differently from G2 liquids, for BL-H samples, the high concentrations of TPC and MAP found in the low volumes of C1 liquid corresponded to approx. 30-35% of TPC and approx. 55% of MAP found in the total extract from purées. In contrast, for H-BL samples, the lower amount of TPC and MAP found in higher volumes of C1 liquid corresponded to approx. 40% of TPC and approx. 25-35% of MAP found in the total extracts from purées.

Hence, if the sum of gravitational and entrapped /easily bound liquid fractions (C1) is taken into consideration, both the concentration of MAP and the rate of these compounds released from the matrix were enhanced in C1 by a preliminary BL-step (BLH samples). These effects were less pronounced on TPC.

With reference to %PC, it decreased with BL-time, as MAP and TPC increased in the liquid samples, underlying the involvement of oxidative phenomena in this trend. Besides, the great differences produced in MAP and TPC values by the operation order corresponded to small differences in %PC, because they were more related to syneresis than to oxidative phenomena.

5.2.1.3. Phenolics of particle-bound fraction C3

Particle-bound phenolic compounds were extracted from the purée pellets remaining after the removal of soluble and loosely associated phenolics, using an acetone/formic acid extracting solution (C3).
In Figure 5.4 average TPC and MAP values of the C3 extracts, referred to 100 g of purée product, are reported.

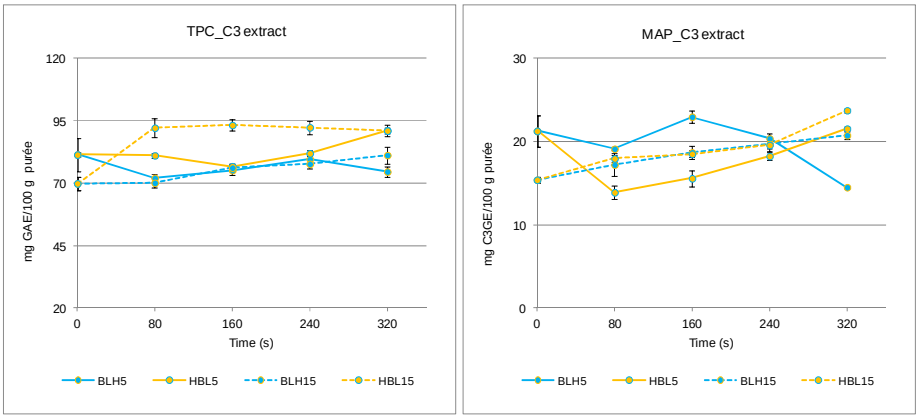


Figure 5.4: Total phenolic compounds (TPC, mg GAE/100g) and monomeric anthocyanin pigments (MAP, mg C3GE/100 g) of the C3 extract of blueberry purées as a function of blanching (BL) time, homogenization (H) time and operation order (BL+H; H+BL)**.**
(Average of the replicates ± std error).

Multifactorial analysis of variance indicates that in C3, TPC content was mainly influenced by operation order and secondly by BL-time, while H-time had significant effect only in the interaction with BL-time. Besides, MAP content depended only on BL-time and it was modulated by the interaction between main factors. Both BL-time and operation order influenced %PC which was also modulated by the interaction between the two factors (Table 5.1).

In fact, if the average values of the C3 extract referred to 100 g of purée product (Figure 5.4) are analysed, in unblanched purées (BL=0s) TPC were 69.8 and 81.4 mg GAE/100 g in H15 and H5, respectively. In thermally treated purées, these values increased in extensively blended samples (H15) and this increase was more pronounced when the blanching step was applied on already homogenate matrices (+32% in H15BL80) (Table 5.5). As a result, whatever the BL-time applied, particle-bound TPC were higher in HBL extracts compared to BLH ones, and the highest values were associated to the H15BL samples.
Besides, particle-bound MAP recovered in C3 from unblanched purées (BL=0) were 15.4 and 21.3 mg GAE/100 g in H15 and H5, respectively. These values increased with BL-time in extensively blended samples (H15), without differences related to the operation order. On the

contrary for H5 purées a decrease of the values was registered from 0 to 80 s BL time in HBL samples and from 240 to 320 s BL time in BLH purées.

On the other hand, if particle-bound phenolics measured in C3 were expressed as a percentage to total phenolics measured in E, and not referred to 100 g of purée, a data trend was evidenced among samples as a function of the operation order and H-time (Figure 5.5). In fact, for TPC, the rate of particle bound to total compounds was always higher in HBL purées compared to BLH ones, whatever the BL-time (40% *vs* 27% for particle bound TPC in HBL *vs* BLH purées, respectively) and higher values were produced by longer H-times (HBL15>HBL5). For MAP this trend was confirmed for BL-time ≥160s (42% *vs* 25% for particle bound MAP in HBL *vs* BLH purées, respectively) with BLH values decreasing with the increase of BL-time.

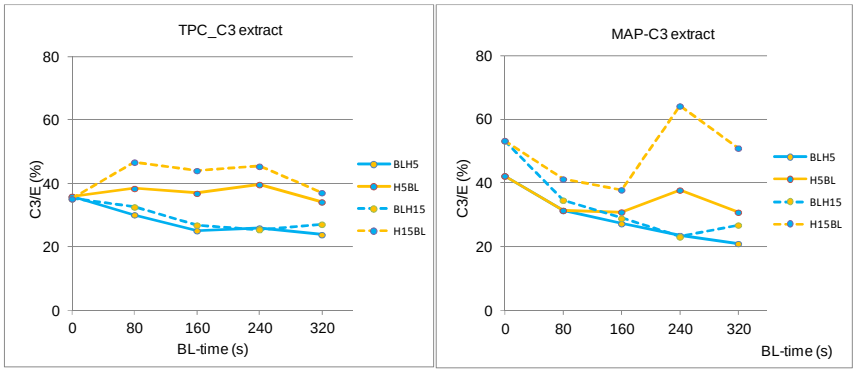


Figure 5.5: Total phenolic compounds (TPC) and monomeric anthocyanin pigments (MAP) of C3 extract from blueberry purées expressed as percentage to TPC and MAP of total extract E in function of blanching (BL) time, homogenization (H) time and operation order (BL+H; H+BL)**.**

Hence, we can conclude that:

- Even though the liquid fraction of purées had been removed and the pellet had been resuspended and rinsed with diluted formic acid, high amount of TPC and MAP compounds remained associated to the cell wall materials (CWM). Indeed, a residual colour persisted in the pellets fibers even after the C3-acetone extraction, with a trend among pellets which was visible at the naked eyes ($H_{5\text{-}15}$ $BL_{80\text{-}240}$>$BL_{80}H_{5\text{-}15}$; $H_{5\text{-}15}BL_{320}$>$BL_{160\text{-}320}H_{5\text{-}15}$).
- A blanching step carried out on already homogenate matrices increased the rate of TPC and MAP compound strongly associated to CWM, compared to a preliminary blanching step operated on raw berries. The interactions between phenolic compounds and CWM were greater in extensively blended products.
- The rate of particle-bound to total MAP and TPC decreased with BL-time in BLH but not in HBL purées.

Both phenolic molecules entrapped into compartments of unbroken cells and phenolic molecules newly bound to CWM concur to draw the particle bound phenolic fraction.
Binding phenomena between anthocyanins/phenolic acids and cell wall materials can occur when these metabolites are released from the plant cell vacuoles due to processing or mastication. These interactions can retard/hinder the release of phenolics but also protect and enhance their transportation to the place of action, hence they are relevant for phenolic bioactivities (Mazza & Kay, 2008; Williamson & Clifford, 2010; Cheynier , 2005).
Few data are available describing these interactions in real food systems and in particular concerning blueberries. Based on literature data, in apples these interactions seem to be

influenced by the pore size and matrix organization and hydrophobic/hydrophilic regions of cell walls but also selective interactions between pectins/cellulose and individual polyphenols can occur (Renard et al., 2001; Le Bourvellec et al., 2004). Studies on binding of polyphenols in purple carrots and plant cell wall analogues (Padayachee et al., 2012a; Padayachee et al., 2012b; Padayachee et al., 2013) revealed that both cellulose and pectins can interact with phenolic acids and anthocyanins, binding increasing with the time of contact between anthocyanins and cell wall materials, following a rapid initial binding phase (direct anthocyanin interactions with cellulose and pectins) and a slower subsequent binding (stacking effect, anthocyanins interact with already bound anthocyanins).

In C3, %PC was significantly lower in BLH products compared to HBL ones for BL-time $80s<x<320s$, without differences related to H-time (8.6% and 13.0% mean values for BLH and HBL respectively) (Table 5.6). Thus a correspondence was found between higher %PC values and lower MAP contents in H5 but not in H15 purées.

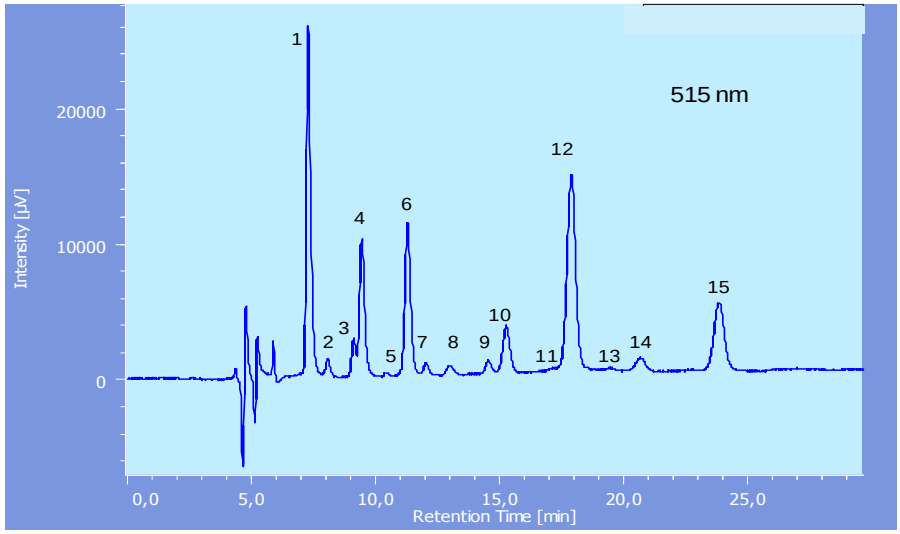
Hence for the blueberry purée systems of this research, it can be inferred that the higher rate of particle bound to total TPC and MAP in HBL *vs* BLH purées could be related to two orders of facts. Firstly, as for other natural dyes, heat can contribute to fix the phenolic compounds on the fruit fibers if they have been previously released from the cell and located on the fibers, and this is the case of HBL but not of BLH products. Second, in BLH purées the deep impact of homogenization on heat-loosened CWM could have altered their surfaces, thus reducing their ability to capture/hold free phenolics.

5.2.2. High Performance Liquid Chromatography

The contribution of individual anthocyanin compounds and chlorogenic acid to the phenolic profile of blueberries and purées was studied more thoroughly by DAD-HPLC. To this aim, total extract E, centrifugal liquid fraction C1 and particle-bound extract C3 of the products were analysed.

Average values for chlorogenic acid (CA) and individual anthocyanins (ACN) of extracts and liquid fraction are reported in Tables 5.8 (E), 5.9 (C1) and 5.10 (C3). Mean values for total ACN as well as for pigments grouped on the basis of the aglycone anthocyanidin or sugar moiety were also included. In Tables 5.7E, 5.7C1 and 5.7C3 results from the multifactorial analysis of variance for these values are reported.

In all the samples, fifteen anthocyanin compounds (ACN) were identified (Figure 5.6) and chlorogenic acid (CA) was detected.



1	D-gal	=	Delphynidin-3-O-galactoside	6	Pt-gal	=	Petunidin-3-O-galactoside	11	Pn-glu	=	Peonidin-3-O-glucoside
2	D-glu	=	Delphinidin-3-O-glucoside	7	C-ara	=	Cyanidin-3-O-arabinoside	12	M-gal	=	Malvidin-3-O-galactoside
3	C-gal	=	Cyanidin-3-O-galactoside	8	Pt-glu	=	Petunidin-3-O-glucoside	13	Pn-ara	=	Peonidin-3-O-arabinoside
4	D-ara	=	Delphynidin-3-O-arabinoside	9	Pn-gal	=	Peonidin-3-O-galactoside	14	M-glu	=	Malvidin-3-O-glucoside
5	C-glu	=	Cyanidin-3-O-glucoside	10	Pt-ara	=	Petunidin-3-O-arabinoside	15	M-ara	=	Malvidin-3-O-arabinoside

Figure 5.6: Chromatogram of anthocyanins from blueberry purée and peaks attribution.

Blueberry fruits (total extract E) were characterized by a total anthocyanin content of 92.72 mg C3GE/100g. Delphinidin and malvidin derivatives were the main anthocyanin compounds present in fruits and made up 75% approx. of total anthocyanin content, followed in the order by petunidines (19.4%), cyanidins (3.7%) and peonidins (1.9%). With respect to the sugar moiety, galactosides covered 66.2% of the total ACNs, followed in the order by arabinosides (30.6%) and glucosides (3.2%).

Total ACN of the berries increased with blanching (BL) time (+27% for ACN, BL≥240s). Besides, homogenization (H) had a strong detrimental impact on total ACN (−39% and −79% in H5 and H15 samples, respectively). Within this data trend, which is in agreement with

spectrophotometric data trend, blueberry delphynidins, and to a less extent petunidins, underwent the greatest losses due to mechanical treatments, with the former reducing their initial content by 65% (H5) and 95% (H15) due to the blending operations.

Total ACN (mg C3GE/100 g) and percent to total ACN (% Total ACN) of purée products (E extracts) in function of BL-time, homogenization time (H) and operation order (BL+H; H+BL) are reported in Figure 5.7.

Blending-blanching operation order was the main processing factor influencing the total ACN content of purées (Table 5.7.E) with BLH products being always characterized, within the same BL-time, by higher ACN values compared to HBL purées (88.6 mg C3GE/100 g and 51.2 C3GE/100 g are average ACN values for BLH and HBL purées, respectively). This trend was mainly determined by delphynidins, which were abundant in the products but quite prone to oxidation in unblanched tissues, as mentioned before. On the contrary, malvidins, the other mostly represented ACN in blueberries, were particularly stable all through the purée processing steps. Hence, despite the initial similar percentage content in berries, in HBL purées after a blanching treatment of 240s, malvidins were 56.4% (H5) and 81.1% (H15) but delphynidins only 17% (H5) and 3.5% (H15).

Hence, processing not blanched (NB) berries (HBL purées) resulted in a significant loss of anthocyanins which has to be related to the decrease of delphinidin derivatives. Direct and indirect (PPO, POD) oxidative reactions occurring in crude, homogenized fruit tissues could account for this degradations of phenolic compounds in NB samples. On the base of the structure-activity relationship, delphinidin and petunidin in fact are considered very reactive anthocyanin compounds (Rahman et al., 2006) and, thus, they could be easily oxidized along processing, as already reported in juice (Lee et al, 2002; Rossi et al., 2003).

Figures 5.8 and 5.9 report total ACN (mg C3GE/100 g) and percent to total ACN (% Total ACN) of C1 liquid fraction (easily accessible compounds) and C3 extract (particle-bound compounds) of purées.

In C1 we observe that, as expected, ACN were much more concentrated in the lower amount of liquid separated by centrifugation from BLH purées then in the liquid from HBL purées. Furthermore, as well as in the whole homogenate matrix, also in the liquid fraction of purées the protective effect of berry blanching on the more unstable delphynidin and petunidin compounds was displayed.

If we analyze particle bound anthocyanins (C3) we observe that in HBL products, 50% approx. of total anthocyanins (E) remained associated to the cell wall materials (20 mg C3GE/100 g in C3 *vs* 40 mg C3GE/100 g in E, approx.). This percentage decreased to 22% approx. in BLH purées (22 mg C3GE/100 g in C3 *vs* 100 mg C3GE/100 g in E, approx.).

If individual anthocyanidins are considered, it can be observed that, whatever the operation order, for each BL-time, the percentage to total ACN of delphynidins was always higher in C3 extract compared to C1 fraction. On the contrary, percentage to total ACN of malvidins was always higher in C1 compared to C3. Hence it can be inferred that the extent of interaction with cell wall materials was greater for delphynidins than for malvidins.

Interactions between individual anthocyanins and carbohydrate polymers are object of investigations due to the nutritional relevance of these phenomena. Differences in the binding capacity of anthocyanins to cell wall material have been reported and related to the acylation pattern (Padayachee et al., 2013) and to the number of hydroxyl groups characterizing the

molecules (Fernandes et al., 2014), binding capacity increasing with the number of hydroxyls (delphynidins>cyanidin).

In figure 5.10, average values for CA (mg C3GE/100 g) of total phenolic extract E, C1 liquid fraction and C3 extract are reported in function of BL-time, H-time and operation order (BL+H; H+BL).
Blueberry fruits (total extract E) were characterized by a chlorogenic acid content of 28.54 mg/100g. CA values of the berries increased with BL-time (+35%, BL≥240s) while homogenization had a detrimental impact on the compound (−83% and −77% in H5 and H15 samples, respectively).
For purées, operation order was the main processing variable influencing both the total content (E) and the liquid (C1) and particle (C3) fractions of this compound (Table 5.7). In fact, whatever the BL-time, CA content was always higher in BLH samples compared to HBL samples. Besides, if particle-bound CA (C3) was expressed as a percentage to total CA (E), and not referred to 100 g of purée, the rate of particle-bound to total compounds was always higher in HBL purées compared to BLH ones. Hence the rate of CA strongly associated to cell wall materials increased if the blanching step was carried out on already homogenate matrices (HBL>BLH), these data following the same trend found for total phenolic compounds (TPC).

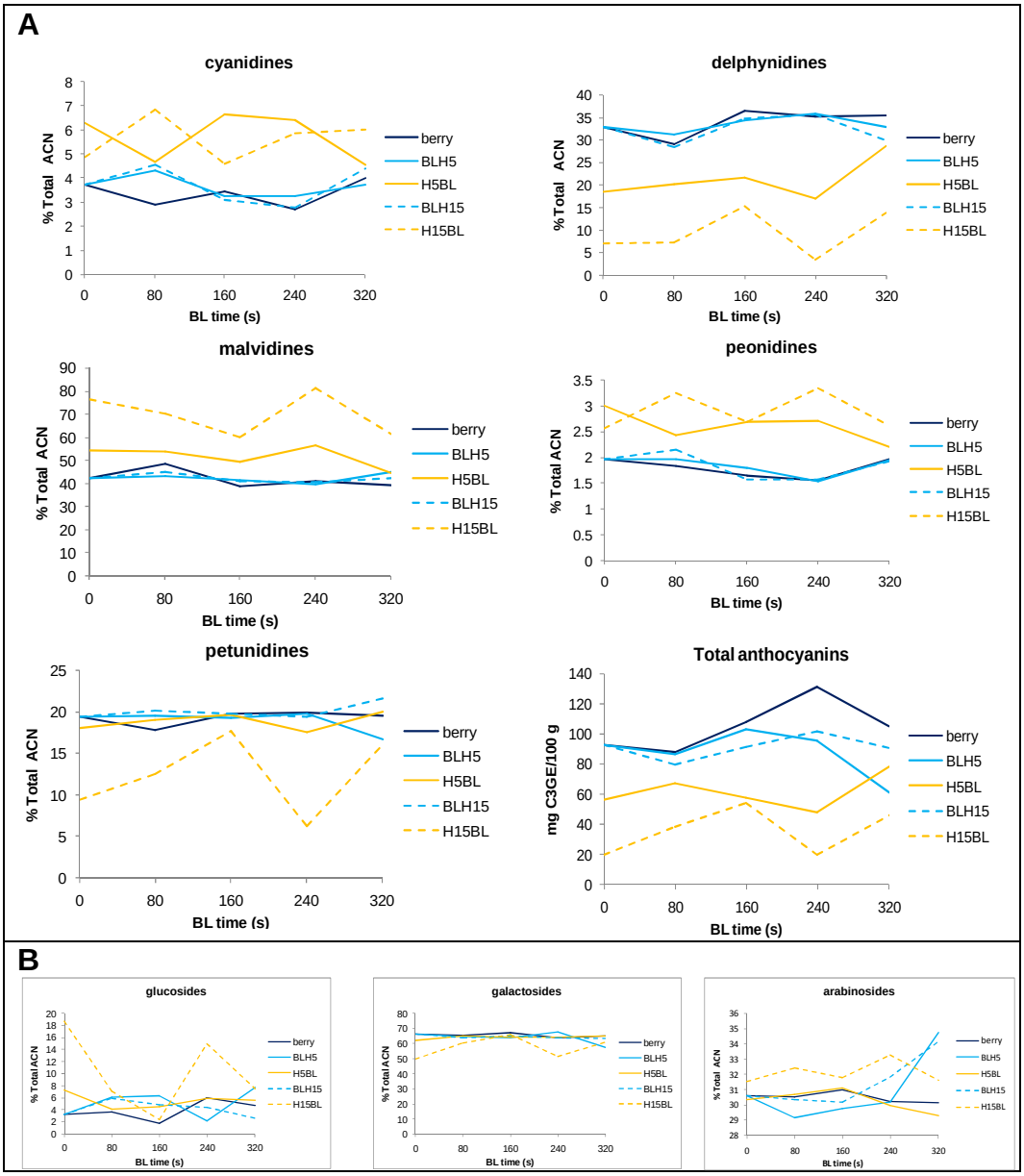


Figure 5.7: Total ACN (mg C3GE/100 g) and percent to total ACN (% Total ACN) of **total phenolic extract E** of blueberry purées in function of BL-time, homogenization time (H) and operation order (BL+H;H+BL).
ACN were grouped on the basis of the aglycone anthocyanidin (A) or sugar moiety (B).
Percent data were computed using the mean concentrations values of Table 5.8)

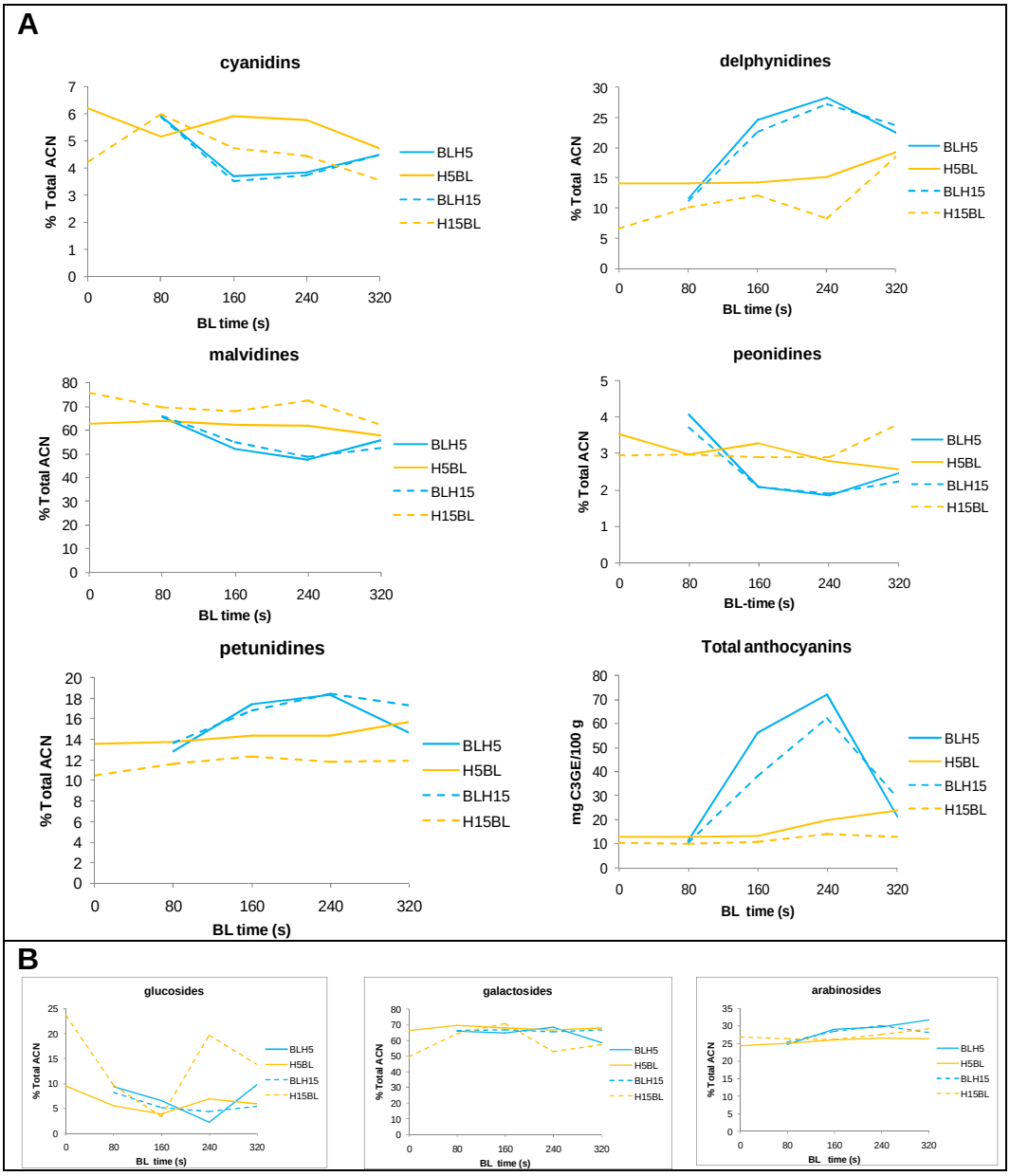


Figure 5.8: Total ACN (mg C3GE/100 g) and percent to total ACN (% Total ACN) of **C1 liquid fraction** of blueberry purées in function of BL-time, homogenization time (H) and operation order (BL+H;H+BL).
ACN were grouped on the basis of the aglycone anthocyanidin (A) or sugar moiety (B).
Percent data were computed using the mean concentrations values of Table 5.9)

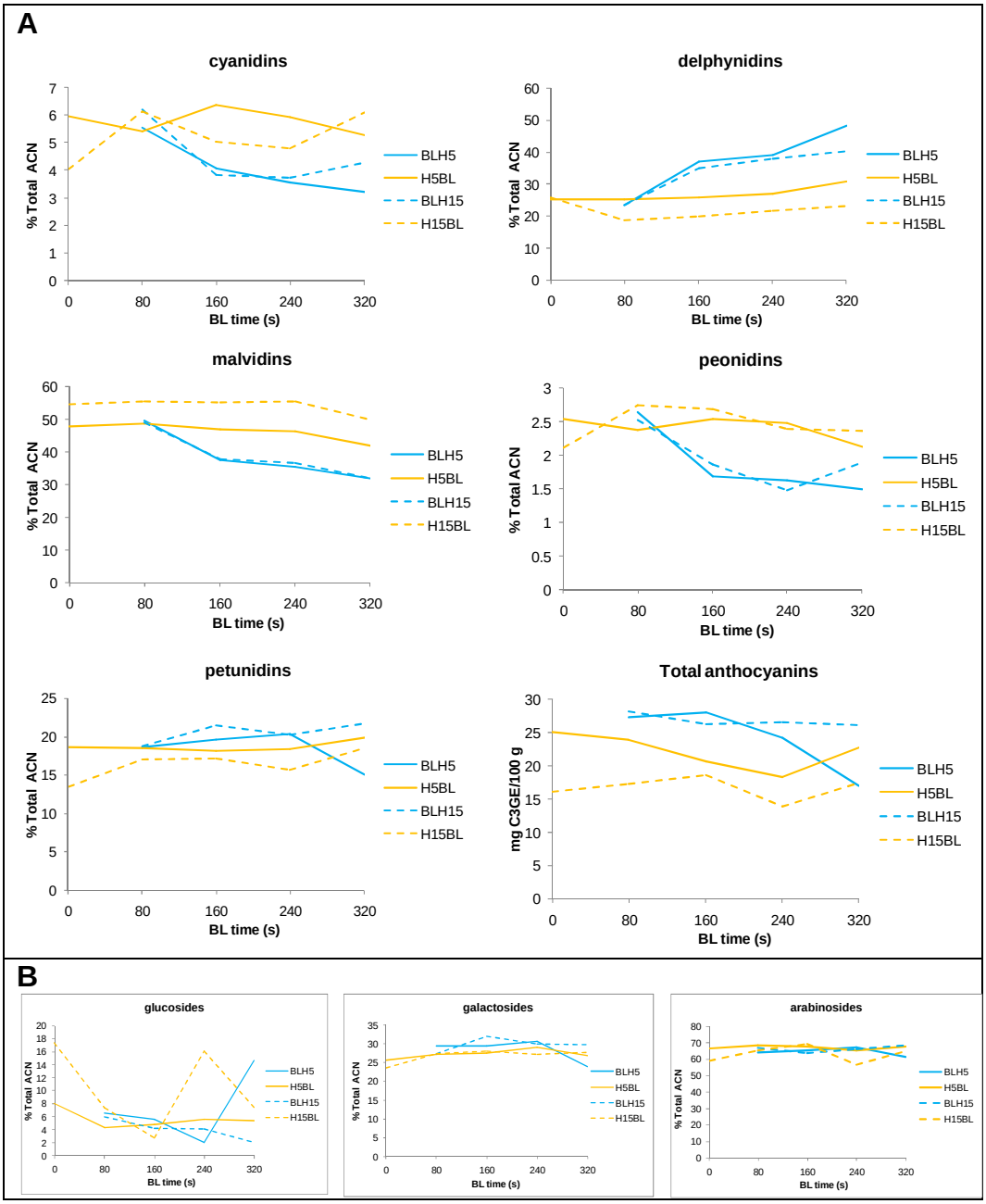


Figure 5.9:.Total ACN (mg C3GE/100 g) and percent to total ACN (% Total ACN) of **C3 extract** of blueberry purées in function of BL-time, homogenization time (H) and operation order (BL+H;H+BL).
ACN were grouped on the basis of the aglycone anthocyanidin (A) or sugar moiety (B).
Percent data were computed using the mean concentrations values of Table 5.10)

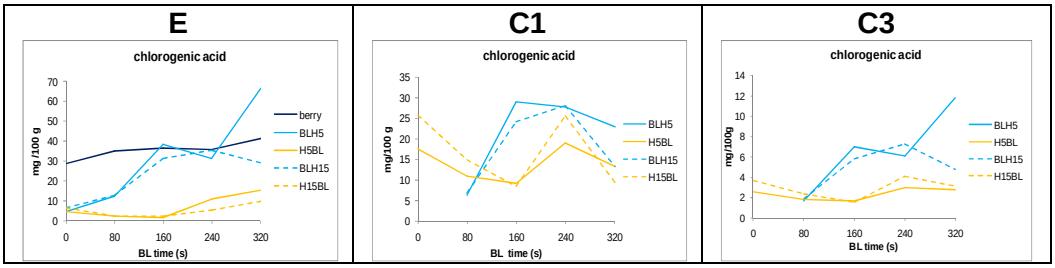


Figure 5.10: Chlorogenic acid (mg/100g) of total phenolic extract E of blueberry fruit and purée products (left), of C1 liquid fractions of blueberry purées (centre) and of C3 extract of blueberry purées (right) in function of BL-time, homogenization time (H) and operation order (BL+H;H+BL)

5.3. Conclusions

Results underlined that in the intact fruit system TPC and MAP did not change with BL, which, on the other hand, could enhance the release of TPC from fruit native compartments and/or protect them from oxidative degradation during the H step. In contrast, the losses of TPC and MAP associated to the initial H step could not be compensated by the successive BL step. In purées higher particle-bound to total TPC and MAP ratios were found for H+BL samples. DAD-HPLC analysis indicated that the higher recovery of anthocyanin compounds in BLH compared to HBL purées was related to the protective effect exerted by preliminary berry blanching towards delphynidins and petunidins, most reactive and unstable anthocyanin compounds. Furthermore, in the particle-liquid homogenate systems, delphynidins were mainly associated to cel wall materials and malvidins to the serum phase.

Table 5.1 Multifactorial analysis of variance for total phenolic compounds TPC (mg GAE/100g), monomeric anthocyanin pigments (MAP, mg C3GE/100 g) and colour indices [colour density (CD), polimeric colour (PC), percentage polymeric colour (%PC) and browning index (BI)].

Source of variation: **A**= Blanching time; **B**= Homogenization time; **C**= Operation order

Source	TPC		MAP		CD		PC		PC%		BI	
	F-Ratio	P-Value	F-Ratio	P-Value	F-Ratio	P-Value	F-Ratio	P-Value	F-Ratio	P-Value	F-Ratio	P-Value
Total phenolic extract (**E**)												
A	28.67	***	12.86	***	740.18	***	14.22	***	154.30	***	116.32	***
B	8.99	**	16.10	***	148.78	***	31.26	***	4.63	*	4.69	*
C	109.88	***	50.72	***	796.31	***	2.77	0.11	147.18	***	288.68	***
Interactions:												
AxB	0.93	0.45	1.01	0.41	55.26	***	2.50	0.08	17.82	***	7.23	***
AxC	14.68	***	8.11	***	164.81	***	15.09	***	61.07	***	71.50	***
BxC	0.18	0.67	0.49	0.49	551.14	***	64.56	***	38.41	***	4.69	*
AxBxC	0.24	0.92	2.73	*	52.96	***	10.77	***	16.98	***	5.52	**
Gravitational liquid fraction (**G2**)												
A	70.14	***	791.92	***	122.04	***	6.52	***	49.69	***	6.58	***
B	0.34	0.56	50.36	***	1.48	0.23	0.99	0.32	1.06	0.31	0.57	0.45
C	119.94	***	1945.04	***	232.59	***	28.25	***	2.72	0.11	9.63	*
Interactions:												
AxB	2.24	0.07	11.40	***	2.19	0.09	2.34	0.07	5.07	**	2.67	*
AxC	25.14	***	331.76	***	52.97	***	8.34	***	1.23	0.31	2.48	0.06
BxC	3.25	0.07	18.59	***	11.07	**	0.27	0.61	1.53	0.22	0.32	0.58
AxBxC	1.80	0.13	14.74	***	4.45	*	1.61	0.19	0.46	0.77	1.73	0.16
Centrifugal liquid fraction (**C1**)												
A	118.61	***	124.36	***	20.57	***	1.13	0.36	27.14	***	1.78	0.15
B	0.02	0.90	4.24	*	0.32	0.57	0.09	0.76	1.03	0.32	0.59	0.45
C	361.36	***	341.26	***	16.95	***	1.32	0.26	34.01	***	9.16	0.00
Interactions:												
AxB	5.79	***	1.29	0.28	0.24	0.92	0.26	0.90	0.50	0.73	1.46	0.23
AxC	52.86	***	52.99	***	4.24	**	0.41	0.80	6.25	***	2.02	0.11
BxC	1.55	0.21	3.04	0.09	0.86	0.36	0.63	0.43	0.00	0.97	0.98	0.33
AxBxC	4.17	*	1.30	0.28	0.50	0.74	0.12	0.97	0.69	0.60	0.36	0.84
Formic acid extract (**C2**) of the purées pellet												
A	99.51	***	160.42	***	206.92	***	100.19	***	63.86	***	23.99	***
B	3.01	0.09	10.62	*	55.53	***	21.73	***	0.98	0.33	13.26	**
C	245.00	***	297.37	***	105.03	***	27.51	***	5.89	*	4.16	0.05
Interactions:												
AxB	2.93	*	10.79	***	39.45	***	6.86	**	6.60	**	5.51	**
AxC	35.84	***	47.92	***	16.90	***	6.49	**	15.50	***	3.55	*
BxC	17.63	***	38.81	***	44.83	***	12.22	**	9.61	**	15.21	***
AxBxC	1.83	0.14	3.74	*	5.08	**	5.54	**	1.59	0.21	4.81	**
Acetone-extract (**C3**) of the purées pellet												
A	4.74	**	6.28	***	0.51	0.73	22.10	***	37.36	***	72.68	***
B	1.70	0.20	0.19	0.66	0.66	0.42	0.36	0.55	0.34	0.56	0.15	0.70
C	42.86	***	1.02	0.32	0.07	0.80	100.80	***	97.22	***	234.33	***
Interactions:												
AxB	5.97	***	15.28	***	9.33	***	3.02	*	10.04	***	2.22	0.08
AxC	3.49	*	12.72	***	3.95	**	11.76	***	13.68	***	28.70	***
BxC	5.76	*	7.09	**	3.69	0.06	4.92	*	0.65	0.42	1.73	0.19
AxBxC	2.31	0.06	5.90	***	2.61	*	4.15	**	0.48	0.75	3.24	*

P-value of F ratio: *P<0.05; **P<0.01; ***P<0.001.

[a] blanching time (BL): 0, 80, 160, 240 and 320 s

[b] homogenization time (H): 5 and 15 s

[c] operation order: H+BL; BL+H

Table 5.2: Total phenolic compounds (TPC, mg GAE/100g), monomeric anthocyanin pigments (MAP, mg C3GE/100 g), and colour indices (colour density, CD; polymeric colour, PC; percent poymeric colour, %PC; browning index, BI) of blueberry fruits (f) and purée products (p) as a function of blanching (BL) time, homogenization (H) time and operation order (BL+H; H+BL) **- Total phenolic extract (E) -**

	BL_time (s)	H time (s)																	
		0 (f)		5 (p)						15 (p)									
				BL+H				H+BL				BL+H				H+BL			
TPC	0	291,90 ± 3,67	ab	226,36 ± 3,78	a	A	*b*	226,36 ± 3,78	ab	A	*b*	198,41 ± 8,10	a	A	*a*	198,41 ± 8,10	a	A	*a*	***c,b,c***
	80	273,21 ± 7,52	a	238,69 ± 11,69	a	A	*a*	210,74 ± 12,93	a	A	*a*	214,64 ± 17,08	a	A	*a*	197,04 ± 13,32	a	A	*a*	***b,ab,a,a,a***
	160	309,23 ± 2,86	b	298,23 ± 8,79	b	B	*a*	207,79 ± 10,88	a	A	*a*	282,59 ± 10,85	b	B	*a*	211,43 ± 9,29	a	A	*a*	***b,b,a,b,a***
	240	319,62 ± 10,70	b	306,28 ± 9,19	b	B	*a*	206,29 ± 12,91	a	A	*a*	304,51 ± 11,78	b	B	*a*	203,13 ± 13,82	a	A	*a*	***b,b,a,b,a***
	320	314,06 ± 10,81	b	311,96 ± 10,59	b	B	*a*	266,19 ± 14,34	b	A	*a*	298,47 ± 7,05	b	B	*a*	244,98 ± 13,16	a	A	*a*	***c,bc,ab,bc,a***
MAP	0	74,63 ± 1,66	a	50,26 ± 4,88	a	A	*a*	50,26 ± 4,88	ab	A	*a*	28,84 ± 8,21	a	A	*a*	28,84 ± 8,21	a	A	*a*	***b,a,a***
	80	79,11 ± 4,71	ab	60,79 ± 6,29	ab	B	*a*	44,04 ± 1,56	a	A	*a*	49,58 ± 2,85	ab	A	*a*	43,43 ± 7,85	a	A	*a*	***b,ab,a,a,a***
	160	91,09 ± 5,96	ab	83,93 ± 7,07	b	B	*b*	50,22 ± 5,55	ab	A	*a*	64,34 ± 4,41	bc	A	*a*	48,78 ± 6,48	a	A	*a*	***c,bc,a,ab,a***
	240	86,50 ± 3,00	ab	86,21 ± 8,27	b	B	*a*	48,15 ± 6,26	a	A	*a*	84,87 ± 6,75	c	B	*a*	30,45 ± 6,69	a	A	*a*	***b,b,a,b,a***
	320	92,81 ± 2,02	b	68,77 ± 6,68	ab	A	*a*	69,65 ± 4,09	b	A	*b*	77,10 ± 3,38	c	B	*a*	46,53 ± 6,13	a	A	*a*	***c,b,b,bc,a***
CD	0	2,30 ± 0,01	a	2,10 ± 0,00	b	A	*a*	2,10 ± 0,00	e	A	*a*	2,07 ± 0,02	c	A	*a*	2,07 ± 0,02	d	A	*a*	***b,a,a***
	80	2,54 ± 0,20	a	1,65 ± 0,00	a	B	*b*	1,21 ± 0,01	a	A	*a*	1,08 ± 0,00	a	A	*a*	1,37 ± 0,01	a	B	*b*	***b,a,a,a,a***
	160	2,93 ± 0,28	a	2,54 ± 0,57	d	B	*b*	1,45 ± 0,01	b	A	*a*	1,66 ± 0,00	b	B	*a*	1,49 ± 0,01	b	A	*a*	***c,bc,a,ab,ab***
	240	2,78 ± 0,17	a	2,52 ± 0,07	cd	B	*a*	1,58 ± 0,01	c	A	*a*	2,19 ± 0,01	d	B	*a*	1,83 ± 0,00	c	A	*a*	***b,b,a,ab,a***
	320	3,01 ± 0,15	a	2,28 ± 0,02	bc	B	*b*	1,93 ± 0,02	d	A	*a*	2,13 ± 0,03	cd	A	*a*	2,13 ± 0,02	d	A	*b*	***b,a,a,a,a***
PC	0	0,22 ± 0,00	a	0,26 ± 0,00	ab	A	*b*	0,26 ± 0,00	b	A	*b*	0,24 ± 0,00	a	A	*a*	0,24 ± 0,00	b	A	*a*	***a,c,b,***
	80	0,18 ± 0,01	a	0,24 ± 0,00	a	B	*a*	0,20 ± 0,01	a	A	*a*	0,22 ± 0,01	a	A	*a*	0,21 ± 0,01	a	A	*a*	***a,b,ab,b,ab***
	160	0,22 ± 0,04	a	0,26 ± 0,01	b	B	*b*	0,23 ± 0,01	ab	B	*a*	0,21 ± 0,01	a	A	*a*	0,25 ± 0,00	b	A	*a*	***a,a,a,a,a***
	240	0,22 ± 0,01	a	0,26 ± 0,00	b	B	*b*	0,23 ± 0,00	ab	A	*a*	0,20 ± 0,00	a	A	*a*	0,25 ± 0,00	b	B	*b*	***a,b,ab,a,b***
	320	0,24 ± 0,03	a	0,23 ± 0,00	a	A	*b*	0,25 ± 0,01	b	A	*a*	0,21 ± 0,00	a	A	*a*	0,25 ± 0,01	b	B	*a*	***a,a,a,a,a***
PC%	0	9,35 ± 0,20	a	12,12 ± 0,05	b	A	*a*	12,12 ± 0,05	a	A	*a*	11,36 ± 0,31	ab	A	*a*	11,36 ± 0,31	a	A	*a*	***a,b,b***
	80	7,23 ± 0,43	a	14,48 ± 0,19	c	A	*a*	16,45 ± 0,82	b	A	*a*	20,72 ± 0,81	c	B	*b*	15,37 ± 0,36	bc	A	*a*	***a,b,b,c,b***
	160	7,29 ± 0,60	a	10,18 ± 0,03	a	A	*a*	16,03 ± 0,59	b	B	*a*	12,46 ± 0,54	b	A	*a*	16,58 ± 0,09	c	B	*a*	***a,b,c,ab,c***
	240	7,97 ± 0,58	a	10,22 ± 0,25	a	A	*a*	14,81 ± 0,26	ab	B	*a*	9,27 ± 0,13	a	A	*a*	13,91 ± 0,16	b	B	*a*	***a,b,c,ab,c***
	320	7,94 ± 0,60	a	9,95 ± 0,12	a	A	*a*	12,69 ± 0,27	a	B	*a*	10,03 ± 0,04	ab	A	*a*	11,52 ± 0,35	a	A	*a*	***a,ab,c,ab,bc***
BI	0	0,04 ± 0,00	a	0,04 ± 0,00	c	A	*a*	0,04 ± 0,00	ab	A	*a*	0,04 ± 0,00	c	A	*a*	0,04 ± 0,00	b	A	*a*	***a,b,b***
	80	0,03 ± 0,00	a	0,05 ± 0,00	d	A	*a*	0,04 ± 0,00	ab	A	*a*	0,04 ± 0,00	c	A	*a*	0,04 ± 0,00	b	A	*a*	***a,b,b,b,b***
	160	0,03 ± 0,00	a	0,04 ± 0,00	b	A	*a*	0,05 ± 0,00	b	B	*a*	0,03 ± 0,00	b	A	*a*	0,05 ± 0,00	b	B	*a*	***a,ab,b,ab,b***
	240	0,03 ± 0,00	a	0,03 ± 0,00	b	A	*b*	0,04 ± 0,00	ab	B	*a*	0,03 ± 0,00	ab	A	*a*	0,04 ± 0,00	b	B	*a*	***a,b,c,ab,c***
	320	0,03 ± 0,00	a	0,03 ± 0,00	a	A	*b*	0,04 ± 0,00	a	B	*b*	0,03 ± 0,00	a	A	*a*	0,04 ± 0,00	a	B	*a*	***a,a,b,a,b***

Values are means ± standard error, n=6
Means follower by different letters are significantly different at P<0.05% (Tukey's test).
(a) small letters in the same column: influence of blanching time within the same product
(A) capital letters within the same row and homogenization time: influence of processing order
(*a*) small letters italics within the same row and processing order: influence of homogenization time
(***a***) small letters italics bold within the same row and blanching time: influence of the product

Table 5.3: Total phenolic compounds (TPC, mg GAE/100g), monomeric anthocyanin pigments (MAP, mg C3GE/100 g), colour density (CD) and colour indices (polymeric colour, PC; percent poymeric colour, %PC; browning index, BI) of purée products as a function of blanching (BL) time, homogenization (H) time and operation order (BL+H; H+BL) – **Gravitational liquid fraction (G2)**-

	BL_time (s)	H time (s) 5 BL+H			H time (s) 5 H+BL			H time (s) 15 BL+H			H time (s) 15 H+BL			
TPC	0	119,44 ± 4,62	a	A *a*	119,44 ± 4,62	a	A *a*	134,24 ± 7,69	a	A *a*	134,24 ± 7,69	b	A *a*	***a,b***
	80	106,96 ± 9,50	a	A *a*	123,07 ± 2,77	a	A *b*	111,24 ± 3,92	a	A *a*	110,73 ± 1,53	a	A *a*	***a,a,a,a***
	160	199,46 ± 6,18	b	B *b*	124,37 ± 3,91	a	A *a*	177,28 ± 1,95	b	B *a*	126,56 ± 3,42	ab	A *a*	***c,a,b,a***
	240	206,70 ± 11,78	b	B *a*	131,70 ± 2,48	a	A *a*	195,65 ± 10,53	b	B *a*	145,23 ± 2,82	bc	A *b*	***b,a,b,a***
	320	222,60 ± 16,96	b	B *a*	155,82 ± 1,62	b	A *a*	199,23 ± 8,05	b	B *a*	156,87 ± 5,23	c	A *a*	***b,a,b,a***
MAP	0	13,16 ± 1,08	a	A *a*	13,16 ± 1,08	a	A *a*	12,38 ± 0,10	a	A *a*	12,38 ± 0,10	a	A *a*	***a,a***
	80	14,26 ± 0,99	a	A *a*	14,96 ± 1,24	a	A *a*	14,34 ± 0,86	a	A *a*	13,15 ± 0,47	a	A *a*	***a,a,a,a***
	160	70,85 ± 1,89	b	B *b*	18,92 ± 0,44	b	A *a*	48,17 ± 2,33	b	B *a*	18,68 ± 0,63	b	A *a*	***c,a,b,a***
	240	75,94 ± 2,05	b	B *a*	23,01 ± 0,79	c	A *a*	70,58 ± 2,20	c	B *a*	23,20 ± 1,18	b	A *a*	***b,a,b,a***
	320	73,38 ± 1,60	b	B *b*	34,90 ± 0,23	d	A *b*	68,50 ± 0,37	c	B *a*	29,32 ± 2,34	c	A *a*	***b,a,b,a***
CD	0	0,21 ± 0,01	a	A *a*	0,21 ± 0,01	a	A *a*	0,24 ± 0,01	a	A *a*	0,24 ± 0,01	ab	A *a*	***a,b***
	80	0,15 ± 0,03	a	A *a*	0,22 ± 0,03	a	A *a*	0,18 ± 0,01	a	A *a*	0,19 ± 0,01	a	A *a*	***a,a,a,a***
	160	0,60 ± 0,01	b	B *b*	0,22 ± 0,02	a	A *a*	0,44 ± 0,05	b	B *a*	0,26 ± 0,00	b	A *a*	***c,a,b,a***
	240	0,65 ± 0,03	b	B *a*	0,24 ± 0,02	a	A *a*	0,58 ± 0,06	b	B *a*	0,27 ± 0,00	b	A *a*	***b,a,b,a***
	320	0,66 ± 0,02	b	B *a*	0,36 ± 0,02	b	A *a*	0,58 ± 0,03	b	B *a*	0,40 ± 0,03	c	A *a*	***b,a,b,a***
PC	0	0,07 ± 0,01	ab	A *a*	0,07 ± 0,01	b	A *a*	0,07 ± 0,00	a	A *a*	0,07 ± 0,00	b	A *a*	***a,a***
	80	0,03 ± 0,01	a	A *a*	0,05 ± 0,00	a	A *a*	0,05 ± 0,00	a	A *a*	0,06 ± 0,01	ab	A *a*	***a,a,a,a***
	160	0,09 ± 0,00	b	B *b*	0,05 ± 0,00	a	A *a*	0,05 ± 0,00	a	A *a*	0,05 ± 0,00	a	A *a*	***b,a,a,a***
	240	0,09 ± 0,01	b	B *a*	0,05 ± 0,00	a	A *a*	0,10 ± 0,02	a	A *a*	0,05 ± 0,00	a	A *a*	***ab,a,b,a***
	320	0,09 ± 0,02	b	B *a*	0,06 ± 0,01	ab	A *a*	0,08 ± 0,01	a	A *a*	0,05 ± 0,00	a	A *a*	***a,a,a,a***
PC%	0	36,38 ± 1,56	c	A *b*	36,38 ± 1,56	b	A *b*	29,46 ± 0,62	b	A *a*	29,46 ± 0,62	b	A *a*	***b,a***
	80	23,17 ± 2,36	b	A *a*	23,32 ± 2,84	a	A *a*	29,81 ± 1,94	b	A *a*	29,50 ± 3,49	b	A *a*	***a,a,a,a***
	160	15,52 ± 0,56	ab	A *b*	21,59 ± 0,69	a	B *a*	12,03 ± 0,88	a	A *a*	17,52 ± 1,34	a	A *a*	***ab,c,a,bc***
	240	14,29 ± 0,36	ab	A *a*	19,74 ± 0,29	a	B *b*	18,30 ± 5,86	ab	A *a*	16,96 ± 0,85	a	A *a*	***a,a,a,a***
	320	13,79 ± 3,32	a	A *a*	16,48 ± 0,95	a	A *a*	14,57 ± 3,39	a	A *a*	12,80 ± 1,33	a	A *a*	***a,a,a,a***
BI	0	0,02 ± 0,00	a	A *a*	0,02 ± 0,00	b	A *a*	0,03 ± 0,00	a	A *a*	0,03 ± 0,00	b	A *a*	***a,a***
	80	0,01 ± 0,00	a	A *a*	0,02 ± 0,00	a	A *a*	0,02 ± 0,00	a	A *a*	0,02 ± 0,00	a	A *a*	***a,a,a,a***
	160	0,03 ± 0,00	a	B *b*	0,02 ± 0,00	a	A *a*	0,02 ± 0,00	a	A *a*	0,02 ± 0,00	a	A *a*	***a,a,a,a***
	240	0,03 ± 0,00	a	B *a*	0,02 ± 0,00	a	A *a*	0,03 ± 0,00	a	A *a*	0,02 ± 0,00	a	A *a*	***a,a,a,a***
	320	0,02 ± 0,00	a	A *a*	0,02 ± 0,00	a	A *a*	0,02 ± 0,00	a	A *a*	0,02 ± 0,00	a	A *a*	***a,a,a,a***

Values are means ± standard error, n=6
Means follower by different letters are significantly different at P<0.05% (Tukey's test).
(a) small letters in the same column: influence of blanching time within the same product
(A) capital letters within the same row and homogenization time: influence of processing order
(*a*) small letters italics within the same row and processing order: influence of homogenization time
(***a***) small letters italics bold within the same row and blanching time: influence of the product

Table 5.4: Total phenolic compounds (TPC, mg GAE/100g), monomeric anthocyanin pigments (MAP, mg C3GE/100 g), colour density (CD) and colour indices (polymeric colour, PC; percent poymeric colour, %PC; browning index, BI) of purée products as a function of blanching (BL) time, homogenization (H) time and operation order (BL+H; H+BL) – **Centrifugal liquid fraction (C1)-**

	BL_time (s)	H time (s) 5 BL+H				H time (s) 5 H+BL				H time (s) 15 BL+H				H time (s) 15 H+BL				
TPC	0	108,73 ± 2,36	a	A	*a*	108,73 ± 2,36	b	A	*a*	115,00 ± 1,53	a	A	*b*	115,00 ± 1,53	b	A	*b*	***a,b***
	80	107,29 ± 3,27	a	A	*a*	103,64 ± 3,40	ab	A	*b*	99,32 ± 2,57	a	B	*a*	92,40 ± 1,33	a	A	*a*	***b,ab,ab,a***
	160	176,52 ± 5,08	bc	B	*b*	97,58 ± 1,98	a	A	*a*	152,55 ± 7,10	b	B	*a*	103,48 ± 3,86	ab	A	*a*	***bc,a,b,a***
	240	167,39 ± 4,91	b	B	*a*	110,19 ± 2,66	b	A	*a*	190,94 ± 7,34	c	B	*b*	116,70 ± 4,39	b	A	*a*	***b,a,c,a***
	320	192,20 ± 5,79	c	B	*a*	130,74 ± 2,82	c	A	*a*	180,69 ± 6,06	c	B	*a*	134,41 ± 6,23	c	A	*a*	***b,a,b,a***
MAP	0	15,28 ± 1,75	a	A	*a*	15,28 ± 1,75	a	A	*a*	15,23 ± 0,64	a	A	*a*	15,23 ± 0,64	a	A	*a*	***a,a***
	80	18,00 ± 0,82	a	B	*b*	14,88 ± 1,02	a	A	*a*	15,40 ± 0,66	a	A	*a*	13,81 ± 0,47	a	A	*a*	***b,a,ab,a***
	160	60,70 ± 3,40	b	B	*b*	16,63 ± 0,88	ab	A	*a*	44,93 ± 0,72	b	B	*a*	16,38 ± 1,13	a	A	*a*	***c,a,b,a***
	240	65,20 ± 5,13	b	B	*a*	21,05 ± 1,24	b	A	*a*	63,28 ± 4,44	c	B	*a*	21,34 ± 1,28	a	A	*a*	***b,a,b,a***
	320	67,07 ± 5,57	b	B	*a*	32,87 ± 0,47	c	A	*a*	63,81 ± 4,83	c	B	*a*	32,01 ± 4,80	b	A	*a*	***b,a,b,a***
CD	0	0,19 ± 0,02	a	A	*a*	0,19 ± 0,02	a	A	*a*	0,20 ± 0,02	a	A	*a*	0,20 ± 0,02	a	A	*a*	***a,a***
	80	0,22 ± 0,01	a	A	*a*	0,25 ± 0,07	a	A	*a*	0,20 ± 0,01	a	A	*a*	0,24 ± 0,06	a	A	*a*	***a,a,a,a***
	160	0,51 ± 0,05	b	B	*a*	0,25 ± 0,05	a	A	*a*	0,40 ± 0,04	b	A	*a*	0,27 ± 0,03	a	A	*a*	***b,a,ab,a***
	240	0,53 ± 0,02	b	A	*a*	0,31 ± 0,11	a	A	*a*	0,56 ± 0,02	c	A	*a*	0,30 ± 0,09	a	A	*a*	***a,a,a,a***
	320	0,62 ± 0,05	b	A	*a*	0,43 ± 0,13	a	A	*a*	0,51 ± 0,06	bc	A	*a*	0,45 ± 0,06	a	A	*a*	***a,a,a,a***
PC	0	0,03 ± 0,00	a	A	*a*	0,03 ± 0,00	a	A	*a*	0,04 ± 0,01	a	A	*a*	0,04 ± 0,01	a	A	*a*	***a,a***
	80	0,04 ± 0,01	a	A	*a*	0,04 ± 0,01	a	A	*a*	0,03 ± 0,00	a	A	*a*	0,04 ± 0,01	a	A	*a*	***a,a,a,a***
	160	0,03 ± 0,00	a	A	*a*	0,04 ± 0,01	a	A	*a*	0,03 ± 0,00	a	A	*a*	0,04 ± 0,01	a	A	*a*	***a,a,a,a***
	240	0,05 ± 0,01	a	A	*a*	0,04 ± 0,01	a	A	*a*	0,05 ± 0,01	a	A	*a*	0,05 ± 0,01	a	A	*a*	***a,a,a,a***
	320	0,05 ± 0,01	a	A	*a*	0,05 ± 0,01	a	A	*a*	0,04 ± 0,01	a	A	*a*	0,05 ± 0,01	a	A	*a*	***a,a,a,a***
PC%	0	18,37 ± 0,87	c	A	*a*	18,37 ± 0,87	b	A	*a*	20,87 ± 2,26	b	A	*a*	20,87 ± 2,26	b	A	*a*	***a,a***
	80	16,73 ± 3,10	bc	A	*a*	16,63 ± 1,23	b	A	*a*	16,50 ± 1,19	ab	A	*a*	19,10 ± 1,40	b	A	*a*	***a,a,a,a***
	160	6,48 ± 0,12	a	A	*a*	18,10 ± 0,86	b	B	*b*	8,03 ± 0,43	a	A	*b*	15,64 ± 0,08	ab	B	*a*	***a,c,a,b***
	240	9,30 ± 1,66	ab	A	*a*	15,16 ± 1,44	ab	A	*a*	8,37 ± 2,92	a	A	*a*	16,44 ± 1,72	ab	A	*a*	***a,a,a,a***
	320	7,50 ± 0,84	a	A	*a*	10,94 ± 0,61	a	B	*a*	8,13 ± 2,50	a	A	*a*	10,92 ± 0,91	a	A	*a*	***a,a,a,a***
BI	0	0,01 ± 0,00	a	A	*a*	0,01 ± 0,00	a	A	*a*	0,02 ± 0,00	a	A	*a*	0,02 ± 0,00	a	A	*a*	***a,b***
	80	0,02 ± 0,00	a	A	*a*	0,02 ± 0,00	a	A	*a*	0,02 ± 0,00	a	A	*a*	0,02 ± 0,00	a	B	*b*	***a,a,a,a***
	160	0,01 ± 0,00	a	A	*a*	0,02 ± 0,00	a	B	*a*	0,01 ± 0,00	a	A	*a*	0,02 ± 0,00	a	B	*a*	***a,b,a,b***
	240	0,01 ± 0,00	a	A	*a*	0,02 ± 0,00	a	A	*a*	0,01 ± 0,00	a	A	*a*	0,02 ± 0,00	a	A	*a*	***a,a,a,a***
	320	0,01 ± 0,00	a	A	*a*	0,02 ± 0,00	a	A	*a*	0,01 ± 0,00	a	A	*a*	0,02 ± 0,00	a	A	*a*	***a,a,a,a***

Values are means ± standard error, n=6

Means follower by different letters are significantly different at P<0.05% (Tukey's test).

(a) small letters in the same column: influence of blanching time within the same product

(A) capital letters within the same row and homogenization time: influence of processing order

(*a*) small letters italics within the same row and processing order: influence of homogenization time

(***a***) small letters italics bold within the same row and blanching time: influence of the product

Table 5.5: Total phenolic compounds (TPC, mg GAE/100g), monomeric anthocyanin pigments (MAP, mg C3GE/100 g), colour density (CD) and colour indices (polymeric colour, PC; percent poymeric colour, %PC; browning index, BI) of purée products as a function of blanching (BL) time, homogenization (H) time and operation order (BL+H; H+BL) – F**ormic acid-extract of the pellet (C2)-**

	BL_time (s)	H time (s) 5 BL+H	H time (s) 5 H+BL	H time (s) 15 BL+H	H time (s) 15 H+BL	
TPC	0	29,85 ± 4,95 a A *a*	29,85 ± 4,95 a A *a*	31,35 ± 0,11 a A *a*	31,35 ± 0,11 a A *a*	***a,a***
	80	38,74 ± 3,16 a A *b*	30,24 ± 0,82 a A *a*	29,74 ± 0,68 a A *a*	32,27 ± 2,29 a A *a*	***a,a,a,a***
	160	57,17 ± 1,29 b B *a*	30,10 ± 2,09 a A *a*	53,07 ± 2,09 b B *a*	35,92 ± 0,17 a A *a*	***b,a,b,a***
	240	71,43 ± 0,79 c B *b*	32,97 ± 0,89 ab A *a*	55,83 ± 1,27 b B *a*	33,41 ± 0,92 a A *a*	***c,a,b,a***
	320	69,90 ± 2,22 bc B *a*	44,99 ± 2,21 b A *a*	66,93 ± 2,54 c *a*	47,49 ± 1,86 b A *a*	***b,a,b,a***
MAP	0	10,42 ± 1,45 a A *a*	10,42 ± 1,45 bc A *a*	8,21 ± 0,27 ab A *a*	8,21 ± 0,27 ab A *a*	***b,a***
	80	9,07 ± 0,44 a B *b*	5,16 ± 0,24 a A *a*	5,12 ± 0,20 a A *a*	5,99 ± 0,81 a A *a*	***b,a,a,a***
	160	22,33 ± 0,68 b B *b*	7,79 ± 1,02 ab A *a*	12,95 ± 1,06 b B *a*	8,28 ± 0,13 ab A *a*	***c,a,b,a***
	240	30,55 ± 0,94 c B *b*	9,37 ± 0,47 bc A *a*	22,95 ± 0,94 c B *a*	10,48 ± 0,49 b A *a*	***c,a,b,a***
	320	26,46 ± 0,50 c B *a*	13,21 ± 0,39 c A *a*	28,35 ± 2,50 c A *a*	19,68 ± 1,86 c A *b*	***bc,a,c,ab***
CD	0	0,45 ± 0,02 c A *b*	0,45 ± 0,02 c A *b*	0,26 ± 0,00 b A *a*	0,26 ± 0,00 c A *a*	***b,a,c,ab***
	80	0,14 ± 0,01 a B *b*	0,08 ± 0,01 a A *a*	0,07 ± 0,00 a A *a*	0,10 ± 0,00 a B *a*	***c,ab,a,b***
	160	0,26 ± 0,02 b B *b*	0,09 ± 0,01 a A *a*	0,14 ± 0,01 a A *a*	0,13 ± 0,01 ab A *a*	***b,a,a,a***
	240	0,32 ± 0,02 b B *a*	0,12 ± 0,01 ab A *a*	0,23 ± 0,03 b B *a*	0,16 ± 0,00 b A *a*	***b,a,ab,a***
	320	0,28 ± 0,03 b A *a*	0,18 ± 0,01 b A *a*	0,31 ± 0,01 b B *a*	0,25 ± 0,01 c A *b*	***ab,a,b,ab***
PC	0	0,02 ± 0,00 b A *b*	0,02 ± 0,00 b A *b*	0,02 ± 0,00 c A *a*	0,02 ± 0,00 c A *a*	***b,a***
	80	0,15 ± 0,00 a A *a*	0,01 ± 0,00 a A *a*	0,01 ± 0,00 a A *a*	0,01 ± 0,00 b B *b*	***a,a,a,a***
	160	0,01 ± 0,00 a A *a*	0,01 ± 0,00 a A *a*	0,01 ± 0,00 a A *a*	0,01 ± 0,00 ab A *a*	***a,a,a,a***
	240	0,02 ± 0,00 a B *b*	0,01 ± 0,00 a A *a*	0,01 ± 0,00 a A *a*	0,01 ± 0,00 a A *a*	***b,a,a,a***
	320	0,01 ± 0,00 a A *a*	0,01 ± 0,00 a A *a*	0,02 ± 0,00 b B *a*	0,01 ± 0,00 ab A *a*	***bc,ab,c,a***
PC%	0	5,47 ± 0,08 ab A *a*	5,47 ± 0,08 a A *a*	7,18 ± 0,04 b A *b*	7,18 ± 0,04 ab A *b*	***a,b***
	80	11,00 ± 1,86 b A *a*	9,36 ± 0,72 bc A *a*	14,10 ± 0,63 c A *a*	11,27 ± 0,09 c A *a*	***a,a,a,a***
	160	4,43 ± 0,60 ab A *a*	11,48 ± 0,89 c B *a*	6,32 ± 0,42 ab A *a*	8,78 ± 0,12 bc A *a*	***a,b,a,ab***
	240	4,78 ± 0,61 ab A *a*	7,46 ± 0,29 ab A *b*	4,20 ± 0,35 a A *a*	4,88 ± 0,05 ab A *a*	***a,b,a,ab***
	320	4,92 ± 0,39 a A *a*	5,14 ± 0,27 a A *a*	4,96 ± 0,27 a A *a*	3,63 ± 0,05 a A *a*	***a,a,a,a***
BI	0	0,01 ± 0,00 a A *b*	0,01 ± 0,00 b A *b*	0,00 ± 0,00 c A *a*	0,00 ± 0,00 a A *a*	***b,a***
	80	0,01 ± 0,00 a A *a*	0,00 ± 0,00 a A *a*	0,00 ± 0,00 b A *a*	0,00 ± 0,00 a A *a*	***a,a,a,a***
	160	0,00 ± 0,00 a A *a*	0,00 ± 0,00 a A *a*	0,00 ± 0,00 ab A *a*	0,00 ± 0,00 a B *a*	***a,a,a,a***
	240	0,00 ± 0,00 a B *b*	0,00 ± 0,00 a A *a*	0,00 ± 0,00 a A *a*	0,00 ± 0,00 a A *a*	***b,ab,a,ab***
	320	0,00 ± 0,00 a A *a*	0,00 ± 0,00 a A *a*	0,00 ± 0,00 c A *a*	0,00 ± 0,00 a A *a*	***ab,a,b,ab***

Values are means ± standard error, n=6
Means follower by different letters are significantly different at P<0.05% (Tukey's test).
(a) small letters in the same column: influence of blanching time within the same product
(A) capital letters within the same row and homogenization time: influence of processing order
(*a*) small letters italics within the same row and processing order: influence of homogenization time
(***a***) small letters italics bold within the same row and blanching time: influence of the product

Table 5.6: Total phenolic compounds (TPC, mg GAE/100g), monomeric anthocyanin pigments (MAP, mg C3GE/100 g), colour density (CD) and colour indices (polymeric colour, PC; percent poymeric colour, %PC; browning index, BI) of purée products as a function of blanching (BL) time, homogenization (H) time and operation order (BL+H; H+BL) – **Acetone-extract of the pellet (C3)**

	BL_time (s)	H time (s) 5 BL+H				5 H+BL				15 BL+H				15 H+BL			
TPC	0	81,36 ± 6,51	a	A	*a*	81,36 ± 6,51	a	A	*a*	69,84 ± 2,70	a	A	*a*	69,84 ± 2,70	a	A	*a*	***b,a***
	80	72,00 ± 1,48	a	A	*a*	81,05 ± 1,19	a	B	*a*	70,06 ± 1,78	a	A	*a*	92,13 ± 3,89	b	B	*b*	***ab,b,a,c***
	160	75,13 ± 1,85	a	A	*a*	76,72 ± 2,98	a	A	*a*	76,13 ± 1,54	ab	A	*a*	93,22 ± 2,33	b	B	*b*	***a,a,a,b***
	240	79,59 ± 2,25	a	A	*a*	81,91 ± 3,88	a	A	*a*	77,64 ± 1,95	ab	A	*a*	92,31 ± 2,65	b	B	*a*	***a,ab,a,b***
	320	74,55 ± 2,15	a	A	*a*	91,07 ± 2,96	a	B	*a*	81,16 ± 3,42	c	A	*a*	90,97 ± 2,24	b	B	*a*	***a,b,ab,b***
MAP	0	21,26 ± 1,90	b	A	*b*	21,26 ± 1,90	b	A	*b*	15,38 ± 0,38	a	A	*a*	15,38 ± 0,38	a	A	*a*	***b,a***
	80	19,11 ± 0,23	ab	B	*a*	13,88 ± 0,81	a	A	*a*	17,20 ± 1,34	ab	A	*a*	17,95 ± 0,45	b	A	*b*	***b,a,ab,b***
	160	22,96 ± 0,69	b	B	*b*	15,56 ± 1,00	a	A	*a*	18,67 ± 0,77	abc	A	*a*	18,50 ± 0,42	b	A	*b*	***a,b,b,b***
	240	20,36 ± 0,63	b	B	*a*	18,23 ± 0,48	ab	A	*a*	19,67 ± 0,51	bc	A	*a*	19,56 ± 0,72	b	A	*a*	***a,a,a,a***
	320	14,49 ± 0,18	a	A	*a*	21,53 ± 0,26	b	B	*a*	20,73 ± 0,50	c	A	*b*	23,74 ± 0,25	c	B	*b*	***b,a,a,a***
CD	0	0,76 ± 0,08	b	A	*b*	0,76 ± 0,08	a	A	*b*	0,54 ± 0,01	a	A	*a*	0,54 ± 0,01	a	A	*a*	***b,a***
	80	0,69 ± 0,02	ab	B	*a*	0,56 ± 0,02	a	A	*a*	0,62 ± 0,05	ab	A	*a*	0,67 ± 0,01	b	A	*b*	***a,a,a,a***
	160	0,78 ± 0,04	b	B	*b*	0,58 ± 0,02	a	A	*a*	0,62 ± 0,02	ab	A	*a*	0,66 ± 0,00	b	A	*b*	***b,a,a,ab***
	240	0,69 ± 0,03	ab	A	*a*	0,63 ± 0,01	a	A	*a*	0,66 ± 0,02	b	A	*a*	0,71 ± 0,00	b	A	*b*	***a,a,a,a***
	320	0,49 ± 0,07	a	A	*a*	0,73 ± 0,00	a	A	*a*	0,70 ± 0,02	b	A	*b*	0,79 ± 0,02	c	B	*a*	***a,ab,b,b***
PC	0	0,09 ± 0,00	d	A	*a*	0,09 ± 0,00	a	A	*a*	0,08 ± 0,00	b	A	*a*	0,08 ± 0,00	a	A	*a*	***b,a***
	80	0,08 ± 0,00	cd	A	*a*	0,08 ± 0,00	a	A	*a*	0,08 ± 0,00	b	A	*a*	0,10 ± 0,00	c	B	*b*	***a,a,a,b***
	160	0,07 ± 0,00	bc	A	*b*	0,08 ± 0,00	a	A	*a*	0,05 ± 0,00	a	A	*a*	0,09 ± 0,00	bc	B	*b*	***b,bc,a,c***
	240	0,06 ± 0,00	ab	A	*a*	0,08 ± 0,00	a	A	*a*	0,06 ± 0,00	a	A	*a*	0,08 ± 0,00	ab	B	*a*	***a,b,a,b***
	320	0,05 ± 0,00	a	A	*a*	0,08 ± 0,00	a	B	*a*	0,06 ± 0,00	a	A	*a*	0,08 ± 0,00	a	B	*a*	***a,c,ab,bc***
PC%	0	11,75 ± 0,79	bc	A	*a*	11,75 ± 0,79	a	A	*a*	14,36 ± 0,15	b	A	*b*	14,36 ± 0,02	c	A	*b*	***a,b***
	80	12,13 ± 0,34	c	A	*a*	14,87 ± 0,33	a	B	*a*	13,08 ± 0,89	b	A	*a*	14,52 ± 0,02	c	A	*a*	***a,a,a,a***
	160	8,56 ± 0,21	a	A	*a*	13,39 ± 0,68	a	B	*a*	8,77 ± 0,31	a	A	*a*	13,72 ± 0,04	c	B	*a*	***a,b,a,b***
	240	8,73 ± 0,26	a	A	*a*	13,10 ± 0,49	a	B	*a*	8,48 ± 0,21	a	A	*a*	11,69 ± 0,06	b	B	*a*	***a,a,a,a***
	320	9,98 ± 0,41	ab	A	*b*	11,16 ± 0,30	a	A	*a*	8,16 ± 0,57	a	A	*a*	9,70 ± 0,02	a	A	*a*	***ab,b,a,ab***
BI	0	0,02 ± 0,00	b	A	*a*	0,02 ± 0,00	a	A	*a*	0,01 ± 0,00	b	A	*a*	0,01 ± 0,00	b	A	*a*	***a,a***
	80	0,01 ± 0,00	b	A	*a*	0,02 ± 0,00	a	B	*a*	0,01 ± 0,00	b	A	*a*	0,02 ± 0,00	c	B	*b*	***a,b,a,c***
	160	0,01 ± 0,00	a	A	*b*	0,01 ± 0,00	a	B	*a*	0,01 ± 0,00	a	A	*a*	0,02 ± 0,00	c	B	*b*	***a,b,a,c***
	240	0,01 ± 0,00	a	A	*a*	0,01 ± 0,00	a	B	*a*	0,01 ± 0,00	a	A	*a*	0,01 ± 0,00	b	B	*a*	***a,b,a,b***
	320	0,01 ± 0,00	a	A	*a*	0,01 ± 0,00	a	B	*a*	0,01 ± 0,00	a	A	*a*	0,01 ± 0,00	a	B	*a*	***a,b,a,b***

Values are means ± standard error, n=6
Means follower by different letters are significantly different at P<0.05% (Tukey's test).
(a) small letters in the same column: influence of blanching time within the same product
(A) capital letters within the same row and homogenization time: influence of processing order
(*a*) small letters italics within the same row and processing order: influence of homogenization time
(***a***) small letters italics bold within the same row and blanching time: influence of the product

Table 5.7.E: Multifactorial analysis of variance for individual antocyanin compounds and chlorogenic acid of the total phenolic extract E of purée products as a function of blanching (BL) time, homogenization (H) time and operation order (BL+H; H+BL).

Source of variation	D-gal		D-glu		C-gal		D-ara		C-glu		Pt-gal		C-ara		Pt-glu	
	F-Ratio	P-Value	F-Ratio	P-Value	F-Ratio	P-Value	F-Ratio	P-Value	F-Ratio	P-Value	F-Ratio	P-Value	F-Ratio	P-Value	F-Ratio	P-Value
Main factors:																
A=Blanching time	58.95	***	3.29	*	16.97	***	16.81	***	7.02	**	25.37	***	3.85	*	7.67	***
B=Homogenization time	65.31	***	7.25	*	62.27	***	10.08	**	49.74	***	29.42	***	18.55	***	41.84	***
C=Operation order	454.80	***	45.05	***	0.40	0.54	123.26	***	54.40	***	129.63	***	5.65	*	111.61	***
Interactions:																
AxB	1.84	0.16	2.50	0.08	27.36	***	1.70	0.19	8.49	***	5.60	**	8.45	***	8.86	***
AxC	59.42	***	5.71	**	4.14	*	11.82	***	4.98	**	16.88	***	1.42	0.26	27.28	***
BxC	20.38	***	0.33	0.57	25.66	***	14.44	**	0.10	0.75	31.17	***	13.38	**	6.12	*
AxBxC	6.39	**	1.71	0.19	5.76	**	3.45	*	3.62	*	8.34	***	2.06	0.12	3.81	*

Source of variation	Pn-gal		Pt-ara		Pn-glu		M-gal		Pn-ara		M-glu		M-ara		Ant_tot	
	F-Ratio	P-Value	F-Ratio	P-Value	F-Ratio	P-Value	F-Ratio	P-Value	F-Ratio	P-Value	F-Ratio	P-Value	F-Ratio	P-Value	F-Ratio	P-Value
Main factors:																
A=Blanching time	9.20	***	7.73	***	5.60	**	7.25	***	2.42	0.08	2.91	*	2.72	0.06	11.78	***
B=Homogenization time	11.91	**	9.71	**	38.77	***	9.12	**	8.62	**	0.62	0.44	1.56	0.23	14.74	**
C=Operation order	3.52	0.08	29.95	***	8.21	**	13.26	**	2.77	0.11	0.03	0.85	8.13	**	59.91	***
Interactions:																
AxB	6.36	**	2.22	0.10	6.74	**	4.23	*	6.28	**	2.68	0.06	0.86	0.51	2.45	0.08
AxC	2.47	0.08	4.52	**	1.00	0.43	3.86	*	0.43	0.79	5.29	**	1.45	0.25	8.37	***
BxC	6.79	*	7.85	*	2.82	0.11	4.86	*	1.85	0.19	1.50	0.23	0.45	0.51	8.16	**
AxBxC	2.88	*	1.83	0.16	0.56	0.69	2.55	0.07	2.28	0.10	0.75	0.57	0.58	0.68	2.64	0.06

Source of variation	GAL		ARA		GLU		MALV		DELPH		PETUN		CYAN		PEON		CA	
	F-Ratio	P-Value	F-Ratio	P-Value	F-Ratio	P-Value	F-Ratio	P-Value	F-Ratio	P-Value	F-Ratio	P-Value	F-Ratio	P-Value	F-Ratio	P-Value	F-Ratio	P-Value
Main factors:																		
A=Blanching time	19.89	***	6.96	**	1.11	0.38	4.11	*	31.61	***	17.06	***	42.34	***	8.24	***	219.30	***
B=Homogenization time	25.79	***	5.63	*	4.43	*	4.52	*	30.59	***	22.27	***	207.56	***	32.51	***	59.28	***
C=Operation order	87.64	***	35.34	***	17.50	***	9.71	**	251.09	***	87.22	***	24.05	***	8.43	**	1154.80	***
Interactions:																		
AxB	4.32	*	1.54	0.23	3.68	*	1.94	0.14	1.39	0.27	4.10	*	65.46	***	10.08	***	50.53	***
AxC	14.01	***	4.26	*	7.38	***	2.31	0.09	28.39	***	11.48	***	11.37	***	2.58	0.07	123.94	***
BxC	13.72	**	4.69	*	0.03	0.87	1.99	0.17	15.68	***	20.43	***	77.31	***	8.88	**	25.08	***
AxBxC	4.60	**	1.40	0.27	1.29	0.31	1.29	0.31	4.31	*	5.08	**	13.45	***	3.86	*	33.49	***

D-gal=Delphynidin-3-O-galactoside; D-glu=Delphinidin-3-O-glucoside; C-gal=Cyanidin-3-O-galactoside; D-ara=Delphynidin-3-O-arabinoside; C-glu=Cyanidin-3-O-glucoside; Pt-gal=Petunidin-3-O-galactoside; Pt-glu=Petunidin-3-O-glucoside; C-ara=Cyanidin-3-O-arabinoside; Pn-galPeonidin-3-O-galactoside; Pt-araPetunidin-3-O-arabinoside; M-gal=Malvidin-3-O-galactoside; Pn-glu=Peonidin-3-O-glucoside; M-glu=Malvidin-3-O-glucoside; Pn-ara=Peonidin-3-O-arabinoside; M-ara=Malvidin-3-O-arabinoside; GAL= Total galactosides, ARA=Total arabinosides, GLU=Total glucosides, MALV=Total malvidins, DELPH=Total delphynidins, PETUN=Total Total petunidins, CYAN=Total cyanidins, PEON=Total peonidins, CA=Chlorogenic acid

P-value of F ratio: *P<0.05; **P<0.01; ***P<0.001.

[a] blanching time (BL): 0, 80, 160, 240 and 320 s [b] homogenization time (H): 5 and 15 s [c] operation order: H+BL; BL+H

Table 5.7.C1: Multifactorial analysis of variance for individual antocyanin compounds and chlorogenic acid of the centrifugal liquid fraction C1 of purée products as a function of blanching (BL) time, homogenization (H) time and operation order (BL+H; H+BL).

Source of variation	D-gal		D-glu		C-gal		D-ara		C-glu		Pt-gal		C-ara		Pt-glu	
	F-Ratio	P-Value	F-Ratio	P-Value	F-Ratio	P-Value	F-Ratio	P-Value	F-Ratio	P-Value	F-Ratio	P-Value	F-Ratio	P-Value	F-Ratio	P-Value
Main factors:																
A=Blanching time	256.86	***	21.72	***	93.73	***	297.28	***	15.41	***	393.36	***	60.03	***	29.85	***
B=Homogenization time	48.06	***	1.34	0.26	63.35	***	53.31	***	11.71	**	91.88	***	20.20	***	4.46	*
C=Operation order	690.40	***	57.66	***	200.01	***	856.73	***	67.11	***	1040.22	***	137.19	***	56.39	***
Interactions:																
AxB	8.82	***	5.61	**	5.35	**	6.22	**	26.04	***	9.59	***	2.82	0.05	23.50	***
AxC	237.41	***	27.07	***	58.26	***	259.24	***	36.60	***	326.60	***	35.10	***	30.89	***
BxC	0.00		2.59	0.12	6.36	*	2.45	0.13	3.88	0.06	4.03	0.06	0.18	0.67	3.36	0.08
AxBxC	14.81	***	3.54	*	9.80	***	9.04	***	6.17	**	31.67	***	3.53	*	4.96	**

Source of variation	Pn-gal		Pt-ara		Pn-glu		M-gal		Pn-ara		M-glu		M-ara		Ant_tot	
	F-Ratio	P-Value	F-Ratio	P-Value	F-Ratio	P-Value	F-Ratio	P-Value	F-Ratio	P-Value	F-Ratio	P-Value	F-Ratio	P-Value	F-Ratio	P-Value
Main factors:																
A=Blanching time	47.38	***	150.20	***	4.86	**	144.79	***	76.51	***	56.91	***	109.87	***	259.57	***
B=Homogenization time	15.76	***	16.62	***	9.88	**	36.62	***	20.43	***	68.53	***	9.94	**	44.96	***
C=Operation order	87.09	***	398.69	***	63.91	***	324.15	***	177.33	***	26.75	***	251.14	***	648.67	***
Interactions:																
AxB	3.74	*	4.04	*	5.64	**	2.63	0.07	3.12	*	74.27	***	3.02	*	6.34	**
AxC	32.69	***	124.33	***	7.81	***	108.90	***	75.91	***	48.43	***	79.01	***	203.71	***
BxC	0.43	0.52	0.02	0.88	1.90	0.18	1.10	0.31	0.13	0.72	33.76	***	0.21	0.65	0.02	0.89
AxBxC	1.77	0.17	6.40	**	0.76	0.56	11.11	***	3.87	*	2.74	0.06	5.00	**	15.04	***

Source of variation	GAL		ARA		GLU		MALV		DELPH		PETUN		CYAN		PEON		CA	
	F-Ratio	P-Value	F-Ratio	P-Value	F-Ratio	P-Value	F-Ratio	P-Value	F-Ratio	P-Value	F-Ratio	P-Value	F-Ratio	P-Value	F-Ratio	P-Value	F-Ratio	P-Value
Main factors:																		
A=Blanching time	310.89	***	197.33	***	60.11	***	131.22	***	278.24	***	292.93	***	90.36	***	59.54	***	92.69	***
B=Homogenization time	75.10	***	25.13	***	24.52	***	18.55	***	50.78	***	49.87	***	53.36	***	27.10	***	2.68	0.12
C=Operation order	757.57	***	499.85	***	104.74	***	299.14	***	771.63	***	770.21	***	210.04	***	158.20	***	72.52	***
Interactions:																		
AxB	7.76	***	4.68	**	70.28	***	3.13	*	7.98	***	7.35	***	4.16	*	3.01	*	21.81	***
AxC	250.61	***	152.99	***	63.27	***	94.62	***	252.65	***	240.55	***	58.18	***	45.75	***	51.65	***
BxC	1.57	0.23	0.42	0.53	29.42	***	0.01	0.94	0.42	0.53	1.08	0.31	2.48	0.13			12.57	**
AxBxC	22.16	***	7.89	***	4.73	**	8.10	***	13.06	***	18.87	***	8.09	***	2.66	0.06	1.00	0.43

D-gal=Delphynidin-3-O-galactoside; D-glu=Delphinidin-3-O-glucoside; C-gal=Cyanidin-3-O-galactoside; D-ara=Delphynidin-3-O-arabinoside; C-glu=Cyanidin-3-O-glucoside; Pt-gal=Petunidin-3-O-galactoside; Pt-glu=Petunidin-3-O-glucoside; C-ara=Cyanidin-3-O-arabinoside; Pn-galPeonidin-3-O-galactoside; Pt-araPetunidin-3-O-arabinoside; M-gal=Malvidin-3-O-galactoside; Pn-glu=Peonidin-3-O-glucoside; M-glu=Malvidin-3-O-glucoside; Pn-ara=Peonidin-3-O-arabinoside; M-ara=Malvidin-3-O-arabinoside; GAL= Total galactosides, ARA=Total arabinosides, GLU=Total glucosides, MALV=Total malvidins, DELPH=Total delphynidins, PETUN=Total Total petunidins, CYAN=Total cyanidins, PEON=Total peonidins, CA=Chlorogenic acid

P-value of F ratio: *P<0.05; **P<0.01; ***P<0.001.

[a] blanching time (BL): 0, 80, 160, 240 and 320 s [b] homogenization time (H): 5 and 15 s [c] operation order: H+BL; BL+H

Table 5.7.C3: Multifactorial analysis of variance for individual antocyanin compounds and chlorogenic acid of the particle bound extract C3 of purée products as a function of blanching (BL) time, homogenization (H) time and operation order (BL+H; H+BL).

Source of variation	D-gal		D-glu		C-gal		D-ara		C-glu		Pt-gal		C-ara		Pt-glu	
	F-Ratio	P-Value	F-Ratio	P-Value	F-Ratio	P-Value	F-Ratio	P-Value	F-Ratio	P-Value	F-Ratio	P-Value	F-Ratio	P-Value	F-Ratio	P-Value
Main factors:																
A=Blanching time	53.83	***	74.95	***	197.80	***	81.43	***	137.89	***	26.05	***	52.59	***	77.83	***
B=Homogenization time	76.33	***	286.21	***	205.82	***	102.70	***	128.00	***	57.06	***	142.55	***	0.12	0.73
C=Operation order	906.89	***	385.13	***	0.54	0.47	577.90	***	6.13	*	150.20	***	12.14	**	25.57	***
Interactions:																
AxB	1.81	0.17	127.10	***	246.00	***	39.26	***	61.98	***	59.11	***	63.20	***	60.90	***
AxC	88.50	***	77.33	***	76.31	***	72.72	***	45.42	***	30.57	***	43.34	***	39.81	***
BxC	94.87	***	55.41	***	348.04	***	105.06	***	15.13	***	108.78	***	80.18	***	44.33	***
AxBxC	15.51	***	48.07	***	30.59	***	29.67	***	8.02	***	29.83	***	12.54	***	28.17	***

Source of variation	Pn-gal		Pt-ara		Pn-glu		M-gal		Pn-ara		M-glu		M-ara		Ant_tot	
	F-Ratio	P-Value	F-Ratio	P-Value	F-Ratio	P-Value	F-Ratio	P-Value	F-Ratio	P-Value	F-Ratio	P-Value	F-Ratio	P-Value	F-Ratio	P-Value
Main factors:																
A=Blanching time	29.60	***	75.65	***	34.86	***	143.46	***	24.44	***	449.03	***	31.04	***	45.52	***
B=Homogenization time	24.67	***	26.80	***	18.00	***	82.48	***	1.72	0.20	217.04	***	0.24	0.63	102.20	***
C=Operation order	2.74	0.11	532.18	***	0.78	0.39	7.51	*	0.53	0.47	9.60	**	12.22	**	296.26	***
Interactions:																
AxB	32.37	***	54.56	***	5.99	**	76.50	***	11.14	***	263.49	***	7.20	***	71.10	***
AxC	7.63	***	86.56	***	11.82	***	57.25	***	3.46	*	122.00	***	11.10	***	53.30	***
BxC	24.67	***	186.91	***	2.00	0.17	70.73	***	7.68	*	169.29	***	6.29	*	138.78	***
AxBxC	7.28	***	25.58	***	0.26	0.90	26.56	***	3.90	*	50.84	***	2.03	0.13	32.52	***

Source of variation	GAL		ARA		GLU		MALV		DELPH		PETUN		CYAN		PEON		CA	
	F-Ratio	P-Value	F-Ratio	P-Value	F-Ratio	P-Value	F-Ratio	P-Value	F-Ratio	P-Value	F-Ratio	P-Value	F-Ratio	P-Value	F-Ratio	P-Value	F-Ratio	P-Value
Main factors:																		
A=Blanching time	45.38	***	30.53	***	509.86	***	91.00	***	87.87	***	28.74	***	215.23	***	32.24	***	837.71	***
B=Homogenization time	141.34	***	28.41	***	11.35	**	14.92	**	154.17	***	60.41	***	323.56	***	22.73	***	37.85	***
C=Operation order	351.10	***	215.28	***	32.62	***	8.04	*	1275.90	***	289.21	***	6.73	*	1.83	0.19	3190.35	***
Interactions:																		
AxB	67.66	***	27.74	***	284.58	***	22.00	***	11.50	***	56.10	***	232.89	***	20.51	***	326.00	***
AxC	54.27	***	29.17	***	155.88	***	31.43	***	130.60	***	43.25	***	106.28	***	10.45	***	661.19	***
BxC	175.76	***	60.66	***	198.13	***	20.88	***	142.27	***	130.40	***	279.29	***	16.45	***	352.02	***
AxBxC	41.06	***	13.40	***	81.30	***	8.32	***	25.22	***	25.85	***	27.44	***	3.74	*	244.34	***

D-gal=Delphynidin-3-O-galactoside; D-glu=Delphinidin-3-O-glucoside; C-gal=Cyanidin-3-O-galactoside; D-ara=Delphynidin-3-O-arabinoside; C-glu=Cyanidin-3-O-glucoside; Pt-gal=Petunidin-3-O-galactoside; Pt-glu=Petunidin-3-O-glucoside; C-ara=Cyanidin-3-O-arabinoside; Pn-galPeonidin-3-O-galactoside; Pt-araPetunidin-3-O-arabinoside; M-gal=Malvidin-3-O-galactoside; Pn-glu=Peonidin-3-O-glucoside; M-glu=Malvidin-3-O-glucoside; Pn-ara=Peonidin-3-O-arabinoside; M-ara=Malvidin-3-O-arabinoside; GAL= Total galactosides, ARA=Total arabinosides, GLU=Total glucosides, MALV=Total malvidins, DELPH=Total delphynidins, PETUN=Total Total petunidins, CYAN=Total cyanidins, PEON=Total peonidins, CA=Chlorogenic acid

P-value of F ratio: *P<0.05; **P<0.01; ***P<0.001.

[a] blanching time (BL): 0, 80, 160, 240 and 320 s [b] homogenization time (H): 5 and 15 s [c] operation order: H+BL; BL+H

Table 5.8: Individual antocyanin compounds (mgC3GE/100g) and chlorogenic acid (mg/100g) of the total phenolic extract E of blueberry fruits (f) and purée products (p) as a function of blanching (BL) time, homogenization (H) time and operation order (BL+H; H+BL).

	BL_ time (s)	H time (s)							BL_ time (s)	H time (s)					
		0 (f)	5 (p)			15 (p)				0 (f)	5 (p)			15 (p)	
			BL+H		H+BL	BL+H	H+BL				BL+H	H+BL		BL+H	H+BL
D_gal	0	19.01 ± 1.66 ab	5.91 ± 0.11 a		5.91 ± 0.11 a	0.67 ± 0.15 a	0.67 ± 0.15 a		Pn-gal	0	1.01 ± 0.04 a	0.88 ± 0.04 a		0.88 ± 0.04 a	0.25 ± 0.08 a	0.25 ± 0.08 a
	80	16.33 ± 1.25 a	17.45 ± 0.34 bc		7.68 ± 0.26 a	13.82 ± 1.87 b	1.46 ± 0.10 ab			80	0.93 ± 0.11 a	1.07 ± 0.16 a		1.11 ± 0.06 a	1.09 ± 0.19 b	0.80 ± 0.08 ab
	160	25.58 ± 0.19 c	21.95 ± 2.91 c		7.45 ± 0.35 a	20.01 ± 1.24 bc	4.69 ± 0.84 c			160	1.24 ± 0.01 a	1.09 ± 0.19 a		0.91 ± 0.11 a	0.93 ± 0.13 ab	0.98 ± 0.15 b
	240	28.43 ± 0.12 c	22.30 ± 0.32 c		4.80 ± 0.87 a	22.34 ± 0.96 c	0.30 ± 0.09 a			240	1.36 ± 0.02 a	1.03 ± 0.10 a		0.88 ± 0.21 a	1.03 ± 0.04 b	0.40 ± 0.01 ab
	320	23.83 ± 0.21 bc	11.43 ± 1.60 ab		14.06 ± 0.72 b	13.90 ± 1.41 b	3.44 ± 0.59 bc			320	1.37 ± 0.13 a	0.52 ± 0.06 a		1.00 ± 0.06 a	1.07 ± 0.01 b	0.78 ± 0.17 ab
D-glu	0	0.88 ± 0.10 ab	0.53 ± 0.07 a		0.53 ± 0.07 a	0.20 ± 0.06 a	0.20 ± 0.06 a		Pt-ara	0	5.14 ± 0.23 ab	3.08 ± 0.20 a		3.08 ± 0.20 a	0.58 ± 0.13 a	0.58 ± 0.13 a
	80	0.71 ± 0.23 ab	1.21 ± 0.45 a		0.46 ± 0.05 a	0.95 ± 0.34 ab	0.12 ± 0.02 a			80	4.43 ± 0.92 a	4.74 ± 1.06 a		4.10 ± 0.30 a	4.53 ± 0.98 ab	1.81 ± 0.31 a
	160	0.43 ± 0.29 a	1.97 ± 0.65 a		0.29 ± 0.06 a	1.26 ± 0.34 ab	0.15 ± 0.06 a			160	6.16 ± 0.12 ab	5.42 ± 1.37 a		3.48 ± 0.22 a	4.95 ± 0.93 ab	3.27 ± 0.88 a
	240	2.85 ± 0.06 c	0.67 ± 0.15 a		0.33 ± 0.06 a	1.52 ± 0.05 b	0.12 ± 0.05 a			240	7.33 ± 0.10 b	5.25 ± 0.44 a		2.62 ± 0.78 a	5.73 ± 0.14 b	0.54 ± 0.03 a
	320	1.54 ± 0.04 b	1.26 ± 0.17 a		0.94 ± 0.25 a	0.63 ± 0.15 ab	0.31 ± 0.06 a			320	5.67 ± 0.16 ab	3.17 ± 0.72 a		4.31 ± 0.55 a	5.39 ± 1.19 b	2.39 ± 0.56 a
C-gal	0	2.25 ± 0.05 ab	2.08 ± 0.09 b		2.08 ± 0.09 a	0.49 ± 0.03 a	0.49 ± 0.03 a		Pn-glu	0	0.32 ± 0.00 a	0.44 ± 0.03 a		0.44 ± 0.03 a	0.17 ± 0.00 a	0.17 ± 0.00 a
	80	1.55 ± 0.08 a	2.16 ± 0.22 b		1.90 ± 0.02 a	2.17 ± 0.29 b	1.76 ± 0.04 b			80	0.33 ± 0.05 a	0.35 ± 0.05 a		0.25 ± 0.01 a	0.33 ± 0.01 b	0.21 ± 0.06 a
	160	2.50 ± 0.18 b	1.91 ± 0.05 ab		2.55 ± 0.16 a	1.74 ± 0.19 b	1.71 ± 0.08 b			160	0.23 ± 0.02 a	0.38 ± 0.07 a		0.35 ± 0.00 a	0.27 ± 0.04 ab	0.18 ± 0.04 a
	240	1.99 ± 0.02 ab	2.04 ± 0.22 b		2.05 ± 0.13 a	1.43 ± 0.04 ab	0.65 ± 0.13 a			240	0.35 ± 0.00 a	0.18 ± 0.03 a		0.18 ± 0.02 a	0.28 ± 0.02 ab	0.10 ± 0.05 a
	320	2.66 ± 0.03 b	1.10 ± 0.11 a		2.24 ± 0.21 a	2.50 ± 0.25 b	1.78 ± 0.23 b			320	0.20 ± 0.08 a	0.42 ± 0.09 a		0.43 ± 0.13 a	0.25 ± 0.01 ab	0.19 ± 0.01 a
D-ara	0	10.54 ± 0.20 ab	3.98 ± 0.39 a		3.98 ± 0.39 a	0.51 ± 0.12 a	0.51 ± 0.12 ab		M-gal	0	26.89 ± 0.53 a	19.84 ± 1.01 a		19.84 ± 1.01 a	7.40 ± 1.52 a	7.40 ± 1.52 a
	80	8.42 ± 1.25 a	8.38 ± 2.09 a		5.45 ± 0.32 ab	7.89 ± 1.71 b	1.22 ± 0.20 ab			80	28.21 ± 0.65 a	24.05 ± 4.77 a		24.81 ± 1.11 a	23.03 ± 4.35 b	16.45 ± 1.86 a
	160	13.43 ± 0.45 bc	11.30 ± 2.26 a		4.74 ± 0.28 ab	10.60 ± 1.37 b	3.48 ± 0.93 b			160	28.56 ± 0.17 a	27.51 ± 5.38 a		18.65 ± 0.72 a	24.63 ± 2.52 b	22.23 ± 4.50 a
	240	14.79 ± 0.01 c	11.27 ± 0.67 a		3.09 ± 0.75 a	12.35 ± 0.42 b	0.27 ± 0.01 a			240	34.74 ± 0.21 b	25.98 ± 1.33 a		17.70 ± 3.73 a	26.84 ± 0.78 b	8.22 ± 1.96 a
	320	12.04 ± 0.02 bc	7.37 ± 1.29 a		7.55 ± 0.99 b	12.45 ± 1.72 b	2.62 ± 0.75 ab			320	26.94 ± 0.37 a	15.63 ± 3.00 a		23.09 ± 1.64 a	26.05 ± 1.05 b	17.35 ± 3.51 a
C-glu	0	0.15 ± 0.01 a	0.42 ± 0.03 a		0.42 ± 0.03 c	0.17 ± 0.01 a	0.17 ± 0.10 a		Pn-ara	0	0.49 ± 0.18 a	0.36 ± 0.05 a		0.36 ± 0.05 a	0.08 ± 0.02 a	0.08 ± 0.02 a
	80	0.16 ± 0.02 a	0.47 ± 0.10 a		0.25 ± 0.00 ab	0.42 ± 0.06 b	0.15 ± 0.03 a			80	0.34 ± 0.03 a	0.28 ± 0.00 a		0.28 ± 0.01 a	0.31 ± 0.03 ab	0.24 ± 0.07 a
	160	0.08 ± 0.06 a	0.46 ± 0.07 a		0.21 ± 0.01 ab	0.25 ± 0.04 ab	0.10 ± 0.01 a			160	0.31 ± 0.00 a	0.38 ± 0.02 a		0.29 ± 0.02 a	0.25 ± 0.05 ab	0.31 ± 0.07 a
	240	0.22 ± 0.01 a	0.18 ± 0.05 a		0.18 ± 0.05 a	0.36 ± 0.01 ab	0.11 ± 0.04 a			240	0.32 ± 0.00 a	0.27 ± 0.03 a		0.26 ± 0.02 a	0.29 ± 0.01 ab	0.13 ± 0.01 a
	320	0.21 ± 0.02 a	0.55 ± 0.08 a		0.36 ± 0.03 bc	0.26 ± 0.04 ab	0.21 ± 0.01 a			320	0.50 ± 0.02 a	0.24 ± 0.10 a		0.31 ± 0.06 a	0.43 ± 0.12 b	0.24 ± 0.07 a
Pt-gal	0	12.19 ± 0.15 ab	6.32 ± 0.19 a		6.32 ± 0.19 a	0.96 ± 0.18 a	0.96 ± 0.18 a		M-glu	0	1.01 ± 0.06 a	2.02 ± 0.19 a		2.01 ± 0.19 a	2.80 ± 0.68 a	2.80 ± 0.68 a
	80	10.55 ± 1.12 a	11.22 ± 2.03 a		8.25 ± 0.33 ab	10.64 ± 1.68 b	2.80 ± 0.30 ab			80	1.51 ± 0.13 b	2.33 ± 0.47 a		1.40 ± 0.01 a	2.20 ± 0.48 a	2.05 ± 0.26 a
	160	14.80 ± 0.01 bc	13.18 ± 1.99 a		7.40 ± 0.08 a	12.21 ± 0.94 b	6.18 ± 1.10 b			160	0.74 ± 0.05 a	2.52 ± 0.63 a		1.36 ± 0.05 a	1.80 ± 0.10 a	0.72 ± 0.09 a
	240	17.00 ± 0.06 c	13.23 ± 0.32 a		5.50 ± 0.96 a	13.18 ± 0.32 b	0.51 ± 0.15 a			240	2.79 ± 0.07 d	0.79 ± 0.10 a		1.85 ± 0.27 a	1.54 ± 0.02 a	2.44 ± 0.84 a
	320	13.82 ± 0.19 bc	6.37 ± 0.81 a		10.67 ± 0.58 b	13.67 ± 1.27 b	4.57 ± 0.77 b			320	1.94 ± 0.01 c	1.85 ± 0.14 a		1.96 ± 0.12 a	0.78 ± 0.15 a	2.34 ± 0.53 a
C-ara	0	1.04 ± 0.02 a	1.05 ± 0.03 a		1.05 ± 0.03 a	0.30 ± 0.07 a	0.30 ± 0.07 a		M-ara	0	11.19 ± 0.51 a	8.60 ± 0.38 a		8.60 ± 0.38 a	4.73 ± 1.22 a	4.73 ± 1.22 a
	80	0.83 ± 0.17 a	1.08 ± 0.17 a		1.00 ± 0.05 a	1.05 ± 0.22 b	0.73 ± 0.10 a			80	12.70 ± 1.72 ab	10.71 ± 2.58 a		9.76 ± 0.19 a	10.38 ± 2.39 a	8.48 ± 1.42 a
	160	1.13 ± 0.03 a	0.99 ± 0.15 a		1.06 ± 0.17 a	0.85 ± 0.15 ab	0.69 ± 0.14 a			160	12.42 ± 0.20 ab	12.50 ± 3.12 a		8.30 ± 0.63 a	11.01 ± 2.00 a	9.57 ± 2.53 a
	240	1.33 ± 0.03 a	0.90 ± 0.10 a		0.86 ± 0.16 a	1.03 ± 0.02 ab	0.39 ± 0.02 a			240	15.84 ± 0.02 b	11.18 ± 0.71 a		7.63 ± 2.87 a	12.95 ± 0.50 a	5.18 ± 1.55 a
	320	1.36 ± 0.12 a	0.61 ± 0.02 a		0.99 ± 0.14 a	1.23 ± 0.12 b	0.77 ± 0.14 a			320	12.20 ± 0.31 ab	9.80 ± 2.38 a		9.83 ± 1.13 a	11.42 ± 1.17 a	8.51 ± 2.37 a
Pt-glu	0	0.61 ± 0.01 b	0.73 ± 0.02 ab		0.73 ± 0.02 b	0.32 ± 0.08 a	0.32 ± 0.08 ab		Ant_tot	0	92.72 ± 1.03 ab	56.19 ± 2.01 a		56.19 ± 2.01 a	19.60 ± 4.31 a	19.60 ± 4.31 a
	80	0.55 ± 0.06 ab	0.92 ± 0.09 b		0.40 ± 0.01 a	0.80 ± 0.05 c	0.20 ± 0.02 a			80	87.56 ± 7.62 a	86.40 ± 14.11 a		67.07 ± 2.66 a	79.55 ± 14.0 b	38.45 ± 4.78 a
	160	0.38 ± 0.02 a	1.15 ± 0.11 b		0.39 ± 0.08 a	0.88 ± 0.09 c	0.18 ± 0.04 a			160	107.99 ± 0.90 b	102.67 ± 18.71 a		57.41 ± 2.59 a	91.59 ± 9.47 b	54.41 ± 11.1 a
	240	1.61 ± 0.00 d	0.35 ± 0.08 a		0.33 ± 0.07 a	0.77 ± 0.04 bc	0.16 ± 0.00 a			240	130.95 ± 0.17 c	95.57 ± 4.00 a		48.23 ± 10.82 a	101.6 ± 3.23 b	19.50 ± 4.86 a
	320	1.09 ± 0.07 c	0.64 ± 0.14 ab		0.75 ± 0.05 b	0.43 ± 0.05 ab	0.43 ± 0.01 b			320	105.37 ± 1.20 ab	60.94 ± 9.86 a		78.44 ± 5.84 a	90.43 ± 7.88 b	45.90 ± 9.74 a

Values are means ± standard error, n=6

Means follower by different letters are significantly different at P<0.05% (Tukey's test).

(a) small letters in the same column refer to the influence of blanching time within the same product

continue 1

Table 5.8: continue 1

	BL_ time (s)	H_time (s)															
		0 (f)	5 (p)			15 (p)						0 (f)	5 (p)			15 (p)	
			BL+H		H+BL		BL+H		H+BL				BL+H		H+BL		BL+H		H+BL
MALV	0	39.09 ± 0.07 a	30.45 ± 1.58	a	30.45 ± 1.58	a	14.93 ± 3.41	a	14.93 ± 3.41	a	GAL	0	61.34 ± 1.28 ab	35.02 ± 0.80	a	35.02 ± 0.80	ab	9.76 ± 1.95	a	9.76 ± 1.95	a
	80	42.43 ± 2.50 a	37.08 ± 7.82	a	35.97 ± 1.29	a	35.60 ± 7.20	ab	26.98 ± 3.53	a		80	57.51 ± 3.05 a	55.95 ± 7.07	a	43.74 ± 1.76	ab	50.74 ± 7.80	b	23.25 ± 2.36	ab
	160	41.72 ± 0.32 a	42.53 ± 9.13	a	28.31 ± 1.39	a	37.43 ± 4.62	ab	32.52 ± 7.13	a		160	72.68 ± 0.54 c	65.64 ± 10.45	a	36.95 ± 1.09	ab	59.49 ± 4.63	b	35.79 ± 6.67	b
	240	53.36 ± 0.13 b	37.94 ± 2.13	a	27.18 ± 6.87	a	41.33 ± 1.27	b	15.84 ± 4.35	a		240	83.51 ± 0.35 d	64.56 ± 1.83	a	30.92 ± 5.88	a	64.82 ± 2.07	b	10.08 ± 2.33	a
	320	41.08 ± 0.67 a	27.27 ± 5.50	a	34.88 ± 2.89	a	38.24 ± 2.37	ab	28.19 ± 6.40	a		320	68.62 ± 0.38 bc	35.05 ± 5.35	a	51.04 ± 2.79	b	57.19 ± 3.59	b	27.91 ± 5.26	ab
DELPH	0	30.43 ± 1.36 ab	10.41 ± 0.56	a	10.41 ± 0.56	a	1.37 ± 0.32	a	1.37 ± 0.32	a	ARA	0	28.40 ± 0.10 a	17.06 ± 0.88	a	17.06 ± 0.88	a	6.18 ± 1.53	a	6.18 ± 1.53	a
	80	25.46 ± 2.73 a	27.03 ± 2.87	ab	13.58 ± 0.61	a	22.65 ± 3.91	b	2.80 ± 0.28	ab		80	26.73 ± 4.08 a	25.19 ± 5.90	a	20.59 ± 0.86	a	24.15 ± 5.31	ab	12.48 ± 2.10	a
	160	39.44 ± 0.35 cd	35.21 ± 5.82	b	12.47 ± 0.68	a	31.86 ± 2.95	b	8.31 ± 1.82	b		160	33.45 ± 0.80 ab	30.58 ± 6.91	a	17.86 ± 1.30	a	27.65 ± 4.50	b	17.30 ± 4.40	a
	240	46.06 ± 0.09 d	34.23 ± 1.13	b	8.22 ± 1.68	a	36.20 ± 1.42	b	0.68 ± 0.14	a		240	39.61 ± 0.18 b	28.86 ± 1.94	a	14.45 ± 4.56	a	32.34 ± 1.08	b	6.50 ± 1.57	a
	320	37.41 ± 0.44 bc	20.06 ± 2.72	ab	22.54 ± 1.95	b	26.97 ± 3.27	b	6.37 ± 1.40	ab		320	31.77 ± 0.76 ab	21.18 ± 4.51	a	22.98 ± 2.74	a	30.91 ± 4.08	b	14.53 ± 3.88	a
PETUN	0	17.95 ± 0.09 ab	10.12 ± 0.03	a	10.12 ± 0.03	ab	1.85 ± 0.38	a	1.85 ± 0.38	a	GLU	0	2.98 ± 0.15 a	4.12 ± 0.33	a	4.12 ± 0.33	ab	3.67 ± 0.83	a	3.67 ± 0.83	a
	80	15.53 ± 2.10 a	16.88 ± 3.17	a	12.75 ± 0.64	ab	15.97 ± 2.71	b	4.80 ± 0.62	ab		80	3.26 ± 0.48 a	5.26 ± 1.14	a	2.75 ± 0.05	a	4.68 ± 0.91	a	2.72 ± 0.33	a
	160	21.34 ± 0.11 bc	19.74 ± 3.47	a	11.27 ± 0.38	ab	18.03 ± 1.77	b	9.63 ± 1.93	b		160	1.86 ± 0.43 a	6.46 ± 1.38	a	2.59 ± 0.20	a	4.45 ± 0.35	a	1.32 ± 0.07	a
	240	25.95 ± 0.04 c	18.83 ± 0.68	a	8.45 ± 1.81	a	19.68 ± 0.50	b	1.22 ± 0.18	a		240	7.82 ± 0.00 c	2.15 ± 0.24	a	2.86 ± 0.37	a	4.45 ± 0.09	a	2.93 ± 0.97	a
	320	20.58 ± 0.28 ab	10.17 ± 1.39	a	15.73 ± 1.18	b	19.49 ± 2.40	b	7.39 ± 1.34	ab		320	4.98 ± 0.06 b	4.71 ± 0.01	a	4.43 ± 0.32	b	2.34 ± 0.21	a	3.46 ± 0.60	a
CYAN	0	3.44 ± 0.04 b	3.53 ± 0.09	bc	3.53 ± 0.09	ab	0.96 ± 0.11	a	0.96 ± 0.11	a	CA	0	28.54 ± 0.21 a	4.64 ± 0.24	a	4.64 ± 0.24	a	6.55 ± 1.03	a	6.55 ± 1.03	ab
	80	2.54 ± 0.11 a	3.72 ± 0.05	c	3.15 ± 0.07	a	3.64 ± 0.02	c	2.63 ± 0.16	b		80	35.10 ± 1.61 b	12.42 ± 2.20	a	2.21 ± 0.10	a	12.55 ± 1.58	a	2.37 ± 0.06	a
	160	3.71 ± 0.15 bc	3.35 ± 0.17	bc	3.82 ± 0.02	b	2.84 ± 0.01	b	2.49 ± 0.21	b		160	36.45 ± 0.19 bc	38.26 ± 4.19	b	1.44 ± 0.15	a	31.23 ± 0.14	bc	2.34 ± 0.31	a
	240	3.55 ± 0.01 b	3.11 ± 0.08	b	3.09 ± 0.24	a	2.81 ± 0.02	b	1.15 ± 0.15	a		240	35.82 ± 0.77 b	31.24 ± 1.12	b	10.80 ± 1.52	b	35.26 ± 0.86	c	5.32 ± 0.86	ab
	320	4.22 ± 0.12 c	2.26 ± 0.01	a	3.59 ± 0.04	ab	3.99 ± 0.17	c	2.76 ± 0.37	b		320	41.16 ± 1.07 c	66.66 ± 0.57	c	15.27 ± 0.48	c	28.89 ± 1.29	b	9.58 ± 1.65	b
PEON	0	1.82 ± 0.22 a	1.69 ± 0.07	a	1.69 ± 0.07	a	0.50 ± 0.10	a	0.50 ± 0.10	a												
	80	1.60 ± 0.18 a	1.69 ± 0.21	a	1.63 ± 0.05	a	1.71 ± 0.21	b	1.25 ± 0.20	ab												
	160	1.78 ± 0.03 a	1.85 ± 0.14	a	1.55 ± 0.13	a	1.43 ± 0.13	b	1.47 ± 0.05	b												
	240	2.03 ± 0.02 a	1.47 ± 0.15	a	1.31 ± 0.24	a	1.60 ± 0.07	b	0.65 ± 0.06	ab												
	320	2.08 ± 0.07 a	1.18 ± 0.25	a	1.73 ± 0.13	a	1.74 ± 0.00	b	1.20 ± 0.24	ab												

Values are means ± standard error, n=6
Means follower by different letters are significantly different at P<0.05% (Tukey's test).
(a) small letters in the same column refer to the influence of blanching time within the same product

ID: D-gal=Delphynidin-3-O-galactoside; D-glu=Delphinidin-3-O-glucoside; C-gal=Cyanidin-3-O-galactoside; D-ara=Delphynidin-3-O-arabinoside; C-glu=Cyanidin-3-O-glucoside; Pt-gal=Petunidin-3-O-galactoside; Pt-glu=Petunidin-3-O-glucoside; C-ara=Cyanidin-3-O-arabinoside; Pn-galPeonidin-3-O-galactoside; Pt-araPetunidin-3-O-arabinoside; M-gal=Malvidin-3-O-galactoside; Pn-glu=Peonidin-3-O-glucoside; M-glu=Malvidin-3-O-glucoside; Pn-ara=Peonidin-3-O-arabinoside; M-ara=Malvidin-3-O-arabinoside; GAL= Total galactosides, ARA=Total arabinosides, GLU=Total glucosides, MALV=Total malvidins, DELPH=Total delphynidins, PETUN= Total petunidins, CYAN=Total cyanidins, PEON=Total peonidins, CA=Chlorogenic acid

Table 5.9: Individual antocyanin compounds (mgC3GE/100g) and chlorogenic acid (mg/100g) of the centrifugal liquid fraction C1 of purée products as a function of blanching (BL) time, homogenization (H) time and operation order (BL+H; H+BL).

	BL_time (s)	H_time (s) 5 BL+H		H_time (s) 5 H+BL		H_time (s) 15 BL+H		H_time (s) 15 H+BL	
D_gal	0	1.19 + 0.06	a	1.19 + 0.06	a	0.37 + 0.03	a	0.37 + 0.03	a
	80	0.87 + 0.07	a	1.23 + 0.04	a	0.77 + 0.02	a	0.66 + 0.02	a
	160	8.93 + 0.11	b	1.26 + 0.09	a	5.64 + 0.46	b	0.87 + 0.05	ab
	240	13.61 + 0.68	c	2.00 + 0.04	b	10.99 + 0.24	c	0.62 + 0.02	a
	320	2.61 + 0.92	a	3.08 + 0.16	c	4.56 + 0.52	b	1.40 + 0.27	b
D-glu	0	0.12 + 0.01	a	0.12 + 0.01	ab	0.11 + 0.00	ab	0.11 + 0.00	a
	80	0.07 + 0.02	a	0.07 + 0.01	a	0.06 + 0.01	a	0.06 + 0.01	a
	160	0.67 + 0.05	b	0.07 + 0.02	a	0.34 + 0.03	bc	0.05 + 0.02	a
	240	0.32 + 0.07	a	0.12 + 0.04	ab	0.44 + 0.08	c	0.16 + 0.01	a
	320	0.20 + 0.05	a	0.19 + 0.00	b	0.19 + 0.07	abc	0.23 + 0.10	a
C-gal	0	0.52 + 0.03	a	0.52 + 0.03	a	0.25 + 0.03	a	0.25 + 0.03	a
	80	0.45 + 0.05	a	0.46 + 0.02	a	0.41 + 0.01	a	0.40 + 0.02	a
	160	1.31 + 0.01	b	0.56 + 0.03	ab	0.90 + 0.11	b	0.37 + 0.02	a
	240	1.87 + 0.10	c	0.80 + 0.06	c	1.52 + 0.03	c	0.36 + 0.03	a
	320	0.62 + 0.13	a	0.79 + 0.06	bc	0.93 + 0.12	b	0.28 + 0.12	a
D-ara	0	0.50 + 0.05	a	0.50 + 0.05	a	0.21 + 0.03	a	0.21 + 0.03	a
	80	0.38 + 0.05	a	0.49 + 0.03	a	0.32 + 0.01	a	0.27 + 0.03	ab
	160	4.29 + 0.09	c	0.56 + 0.05	a	2.59 + 0.30	b	0.36 + 0.02	b
	240	6.39 + 0.41	d	0.88 + 0.08	b	5.45 + 0.14	c	0.38 + 0.03	b
	320	2.00 + 0.18	b	1.36 + 0.03	c	2.18 + 0.27	b	0.76 + 0.03	c
C-glu	0	0.11 + 0.02	a	0.11 + 0.02	bc	0.10 + 0.01	ab	0.10 + 0.01	bc
	80	0.09 + 0.01	a	0.06 + 0.01	a	0.06 + 0.01	ab	0.07 + 0.01	b
	160	0.25 + 0.01	b	0.06 + 0.01	a	0.11 + 0.01	b	0.02 + 0.01	a
	240	0.11 + 0.03	a	0.07 + 0.01	ab	0.20 + 0.01	c	0.11 + 0.01	c
	320	0.10 + 0.01	a	0.12 + 0.01	c	0.05 + 0.02	a	0.07 + 0.00	bc
Pt-gal	0	1.19 + 0.06	a	1.19 + 0.06	a	0.58 + 0.04	a	0.58 + 0.04	a
	80	1.02 + 0.10	a	1.22 + 0.07	a	0.96 + 0.04	a	0.78 + 0.04	a
	160	6.55 + 0.19	b	1.31 + 0.10	a	4.37 + 0.38	b	0.94 + 0.13	a
	240	9.26 + 0.51	c	1.98 + 0.08	b	7.71 + 0.19	c	0.91 + 0.05	a
	320	2.02 + 0.17	a	2.70 + 0.13	c	3.51 + 0.12	b	0.75 + 0.03	a
C-ara	0	0.17 + 0.03	a	0.17 + 0.03	a	0.10 + 0.02	a	0.10 + 0.02	a
	80	0.15 + 0.04	a	0.14 + 0.01	a	0.15 + 0.03	a	0.13 + 0.01	a
	160	0.52 + 0.01	b	0.17 + 0.03	a	0.34 + 0.07	ab	0.12 + 0.03	a
	240	0.77 + 0.02	c	0.27 + 0.04	a	0.62 + 0.08	b	0.16 + 0.02	a
	320	0.25 + 0.02	a	0.24 + 0.02	a	0.34 + 0.07	ab	0.11 + 0.02	a
Pt-glu	0	0.15 + 0.01	ab	0.15 + 0.01	a	0.25 + 0.02	a	0.25 + 0.02	bc
	80	0.11 + 0.02	a	0.10 + 0.01	a	0.09 + 0.00	a	0.09 + 0.00	ab
	160	0.64 + 0.03	d	0.11 + 0.02	a	0.30 + 0.05	ab	0.04 + 0.01	a
	240	0.28 + 0.03	c	0.17 + 0.03	a	0.56 + 0.01	b	0.33 + 0.03	c
	320	0.22 + 0.00	bc	0.20 + 0.03	a	0.21 + 0.09	a	0.30 + 0.08	bc
Pn-gal	0	0.25 + 0.02	a	0.25 + 0.02	ab	0.15 + 0.02	a	0.15 + 0.02	a
	80	0.21 + 0.02	a	0.23 + 0.02	a	0.21 + 0.02	ab	0.18 + 0.03	a
	160	0.68 + 0.03	b	0.27 + 0.03	abc	0.48 + 0.05	bc	0.20 + 0.02	ab
	240	0.89 + 0.12	b	0.37 + 0.03	c	0.74 + 0.04	c	0.20 + 0.02	ab
	320	0.27 + 0.08	a	0.35 + 0.00	bc	0.41 + 0.09	ab	0.30 + 0.02	b
Pt-ara	0	0.39 + 0.03	a	0.39 + 0.03	a	0.27 + 0.04	a	0.27 + 0.04	a
	80	0.34 + 0.06	a	0.42 + 0.04	ab	0.35 + 0.02	a	0.27 + 0.02	a
	160	2.61 + 0.07	b	0.48 + 0.07	ab	1.71 + 0.20	b	0.34 + 0.03	a
	240	3.64 + 0.35	b	0.69 + 0.05	bc	3.21 + 0.13	c	0.41 + 0.03	a
	320	0.89 + 0.20	a	0.88 + 0.07	c	1.35 + 0.02	b	0.50 + 0.13	a
Pn-glu	0	0.14 + 0.04	a	0.14 + 0.04	a	0.12 + 0.01	a	0.12 + 0.01	ab
	80	0.19 + 0.04	a	0.09 + 0.01	a	0.12 + 0.03	a	0.07 + 0.01	a
	160	0.30 + 0.01	a	0.11 + 0.02	a	0.18 + 0.02	ab	0.06 + 0.00	a
	240	0.20 + 0.02	a	0.10 + 0.01	a	0.24 + 0.01	b	0.15 + 0.01	b
	320	0.18 + 0.04	a	0.14 + 0.01	a	0.14 + 0.02	a	0.10 + 0.03	ab
M-gal	0	5.29 + 0.20	a	5.29 + 0.20	a	3.80 + 0.23	a	3.81 + 0.23	a
	80	4.92 + 0.47	a	5.64 + 0.22	a	4.48 + 0.09	ab	4.28 + 0.02	a
	160	18.78 + 0.58	b	5.55 + 0.54	a	13.77 + 1.10	c	5.09 + 0.22	a
	240	23.34 + 1.02	c	7.96 + 0.71	ab	19.66 + 0.26	d	5.23 + 0.21	a
	320	6.96 + 0.77	a	9.33 + 0.62	b	10.05 + 1.92	bc	4.66 + 0.67	a
Pn-ara	0	0.07 + 0.02	a	0.07 + 0.02	ab	0.04 + 0.01	a	0.04 + 0.01	a
	80	0.06 + 0.01	a	0.06 + 0.01	a	0.06 + 0.01	ab	0.05 + 0.01	a
	160	0.20 + 0.01	b	0.05 + 0.00	a	0.14 + 0.01	c	0.05 + 0.01	a
	240	0.25 + 0.01	b	0.09 + 0.02	ab	0.21 + 0.00	d	0.05 + 0.00	a
	320	0.08 + 0.01	a	0.12 + 0.01	b	0.11 + 0.02	bc	0.10 + 0.01	b
M-glu	0	0.71 + 0.08	a	0.71 + 0.08	bc	1.88 + 0.13	c	1.88 + 0.13	d
	80	0.61 + 0.08	a	0.40 + 0.01	a	0.52 + 0.02	a	0.66 + 0.02	b
	160	1.84 + 0.10	b	0.48 + 0.04	ab	1.00 + 0.09	b	0.20 + 0.02	a
	240	0.73 + 0.07	a	0.93 + 0.06	c	1.32 + 0.01	b	2.01 + 0.06	d
	320	1.39 + 0.17	b	0.77 + 0.05	bc	1.00 + 0.11	b	1.09 + 0.09	c
M-ara	0	2.00 + 0.12	a	2.00 + 0.12	a	2.17 + 0.09	ab	2.17 + 0.09	a
	80	1.88 + 0.22	a	2.05 + 0.06	a	1.75 + 0.05	a	1.85 + 0.04	a
	160	8.68 + 0.45	b	2.17 + 0.17	a	6.03 + 0.55	c	1.90 + 0.08	a
	240	10.22 + 0.68	b	3.30 + 0.25	b	9.22 + 0.19	d	2.84 + 0.15	a
	320	3.55 + 0.02	a	3.70 + 0.23	b	4.26 + 0.65	bc	2.29 + 0.96	a
Ant_tot	0	12.76 + 0.74	a	12.76 + 0.74	a	10.37 + 0.67	a	10.37 + 0.67	a
	80	11.32 + 1.16	a	12.62 + 0.53	a	10.27 + 0.29	a	9.78 + 0.11	a
	160	56.19 + 1.54	b	13.17 + 1.18	a	37.87 + 3.40	b	10.56 + 0.58	a
	240	71.85 + 4.02	c	19.71 + 1.47	b	62.03 + 1.36	c	13.88 + 0.64	a
	320	21.30 + 0.40	a	23.92 + 1.07	b	29.23 + 2.45	b	12.92 + 2.18	a

Values are means ± standard error, n=6 Means follower by different letters are significantly different at P<0.05% (Tukey's test).

continue 1

(a) small letters in the same column refer to the influence of blanching time within the same product

Table 5.9: continue 1

	BL_time (s)	H_time (s) 5 BL+H		5 H+BL		15 BL+H		15 H+BL	
GAL	0	8.43 + 0.36	a	8.43 + 0.36	a	5.14 + 0.33	a	5.14 + 0.33	a
	80	7.45 + 0.70	a	8.78 + 0.37	a	6.82 + 0.16	a	6.28 + 0.11	a
	160	36.24 + 0.90	b	8.95 + 0.78	a	25.16 + 2.10	b	7.47 + 0.44	a
	240	48.96 + 2.42	c	13.11 + 0.91	b	40.61 + 0.74	c	7.31 + 0.32	a
	320	12.47 + 0.11	a	16.23 + 0.95	b	19.44 + 1.72	b	7.38 + 1.11	a
ARA	0	3.12 + 0.24	a	3.12 + 0.24	a	2.78 + 0.18	a	2.78 + 0.18	a
	80	2.80 + 0.38	a	3.15 + 0.15	a	2.61 + 0.09	a	2.56 + 0.00	a
	160	16.27 + 0.61	b	3.41 + 0.31	a	10.79 + 1.11	b	2.75 + 0.14	a
	240	21.27 + 1.46	c	5.22 + 0.43	b	18.68 + 0.52	c	3.83 + 0.23	a
	320	6.76 + 0.37	a	6.29 + 0.22	b	8.21 + 0.48	b	3.76 + 0.13	a
GLU	0	1.22 + 0.15	a	1.22 + 0.15	abc	2.45 + 0.16	bc	2.45 + 0.16	d
	80	1.06 + 0.08	a	0.70 + 0.02	a	0.84 + 0.04	a	0.94 + 0.00	b
	160	3.68 + 0.03	c	0.51 + 0.09	ab	1.93 + 0.20	bc	0.35 + 0.01	a
	240	1.62 + 0.14	ab	1.38 + 0.13	bc	2.74 + 0.10	c	2.74 + 0.10	d
	320	2.08 + 0.08	b	1.41 + 0.09	c	1.58 + 0.25	ab	1.78 + 0.06	c
MALV	0	8.00 + 0.40	a	8.00 + 0.40	a	7.85 + 0.44	ab	7.85 + 0.44	a
	80	7.41 + 0.77	a	8.07 + 0.27	a	6.74 + 0.15	a	6.78 + 0.05	a
	160	29.29 + 1.12	b	8.19 + 0.74	a	20.80 + 1.74	c	7.18 + 0.31	a
	240	34.29 + 1.76	b	12.19 + 1.02	b	30.20 + 0.46	d	10.07 + 0.42	a
	320	11.89 + 0.91	a	13.79 + 0.79	b	15.30 + 2.67	bc	8.03 + 1.71	a
DELPH	0	1.80 + 0.11	a	1.80 + 0.11	a	0.68 + 0.06	a	0.68 + 0.06	a
	80	1.32 + 0.14	a	1.79 + 0.09	a	1.14 + 0.01	a	0.99 + 0.06	ab
	160	13.88 + 0.15	b	1.88 + 0.15	a	8.57 + 0.79	b	1.27 + 0.08	b
	240	20.31 + 1.15	c	2.99 + 0.15	b	16.87 + 0.45	c	1.15 + 0.05	ab
	320	4.80 + 1.14	a	4.63 + 0.19	c	6.91 + 0.72	b	2.39 + 0.20	c

	BL_time (s)	H_time (s) 5 BL+H		5 H+BL		15 BL+H		15 H+BL	
PETUN	0	1.73 + 0.10	a	1.73 + 0.10	a	1.09 + 0.09	a	1.09 + 0.09	a
	80	1.46 + 0.18	a	1.74 + 0.11	a	1.40 + 0.06	a	1.14 + 0.06	ab
	160	9.79 + 0.23	b	1.90 + 0.18	a	6.38 + 0.62	b	1.31 + 0.15	ab
	240	13.18 + 0.89	c	2.84 + 0.16	b	11.47 + 0.32	c	1.65 + 0.11	b
	320	3.13 + 0.36	a	3.77 + 0.05	c	5.06 + 0.22	b	1.54 + 0.08	ab
CYAN	0	0.79 + 0.07	a	0.79 + 0.07	ab	0.44 + 0.05	a	0.44 + 0.05	a
	80	0.67 + 0.09	a	0.65 + 0.04	a	0.61 + 0.02	ab	0.59 + 0.01	a
	160	2.07 + 0.02	b	0.78 + 0.06	ab	1.34 + 0.19	b	0.50 + 0.03	a
	240	2.76 + 0.10	c	1.14 + 0.10	b	2.33 + 0.11	c	0.62 + 0.04	a
	320	0.96 + 0.15	a	1.13 + 0.06	b	1.31 + 0.20	b	0.46 + 0.14	a
PEON	0	0.45 + 0.06	a	0.45 + 0.06	a	0.31 + 0.04	a	0.31 + 0.04	a
	80	0.46 + 0.02	a	0.38 + 0.04	a	0.38 + 0.05	ab	0.29 + 0.04	a
	160	1.16 + 0.03	b	0.43 + 0.05	a	0.79 + 0.08	c	0.31 + 0.02	a
	240	1.33 + 0.13	b	0.55 + 0.04	a	1.18 + 0.04	d	0.40 + 0.03	a
	320	0.53 + 0.06	a	0.61 + 0.01	a	0.65 + 0.08	bc	0.49 + 0.05	a
CA	0	17.49 + 0.54	b	17.49 + 0.54	bc	25.75 + 0.89	b	25.75 + 0.89	c
	80	6.30 + 0.51	a	10.92 + 0.33	a	6.81 + 0.10	a	14.89 + 0.18	b
	160	28.97 + 0.50	c	9.13 + 0.74	a	24.12 + 1.33	b	8.48 + 0.49	a
	240	27.74 + 1.46	c	18.96 + 1.02	c	28.11 + 0.58	b	25.48 + 1.05	c
	320	22.81 + 3.33	bc	13.28 + 1.13	ab	13.21 + 2.67	a	9.40 + 1.21	a

Values are means ± standard error, n=6

Means follower by different letters are significantly different at P<0.05% (Tukey's test).

(a) small letters in the same column refer to the influence of blanching time within the same product

ID: D-gal=Delphynidin-3-O-galactoside; D-glu=Delphinidin-3-O-glucoside; C-gal=Cyanidin-3-O-galactoside; D-ara=Delphynidin-3-O-arabinoside; C-glu=Cyanidin-3-O-glucoside; Pt-gal=Petunidin-3-O-galactoside; Pt-glu=Petunidin-3-O-glucoside; C-ara=Cyanidin-3-O-arabinoside; Pn-galPeonidin-3-O-galactoside; Pt-araPetunidin-3-O-arabinoside; M-gal=Malvidin-3-O-galactoside; Pn-glu=Peonidin-3-O-glucoside; M-glu=Malvidin-3-O-glucoside; Pn-ara=Peonidin-3-O-arabinoside; M-ara=Malvidin-3-O-arabinoside; GAL= Total galactosides, ARA=Total arabinosides, GLU=Total glucosides, MALV=Total malvidins, DELPH=Total delphynidins, PETUN= Total petunidins, CYAN=Total cyanidins, PEON=Total peonidins, CA=Chlorogenic acid

Table 5.10: Individual antocyanin compounds (mgC3GE/100g) and chlorogenic acid (mg/100g) of the particle-bound extract C3 of purée products as a function of blanching (BL) time, homogenization (H) time and operation order (BL+H; H+BL).

	BL_time (s)	H_time (s) 5 BL+H		H_time (s) 5 H+BL		H_time (s) 15 BL+H		H_time (s) 15 H+BL	
D_gal	0	4.10 + 0.04	a	4.10 + 0.04	bc	3.34 + 0.28	a	3.34 + 0.28	b
	80	4.06 + 0.10	a	4.03 + 0.07	bc	4.52 + 0.15	b	2.07 + 0.07	a
	160	6.65 + 0.10	b	3.54 + 0.08	ab	5.91 + 0.09	c	2.54 + 0.07	a
	240	6.13 + 0.09	b	3.19 + 0.21	a	6.58 + 0.15	cd	2.10 + 0.01	a
	320	6.03 + 0.47	b	4.60 + 0.12	c	6.99 + 0.07	d	2.60 + 0.08	ab
D-glu	0	0.38 + 0.01	b	0.38 + 0.01	d	0.25 + 0.01	bc	0.25 + 0.01	d
	80	0.23 + 0.01	a	0.18 + 0.01	b	0.19 + 0.01	ab	0.15 + 0.01	b
	160	0.44 + 0.01	b	0.14 + 0.01	a	0.28 + 0.00	cd	0.05 + 0.00	a
	240	0.21 + 0.04	a	0.15 + 0.00	ab	0.33 + 0.03	d	0.23 + 0.01	d
	320	0.56 + 0.01	c	0.26 + 0.01	c	0.13 + 0.01	a	0.19 + 0.01	c
C-gal	0	0.97 + 0.02	d	0.97 + 0.02	b	0.35 + 0.02	a	0.35 + 0.02	a
	80	0.95 + 0.02	d	0.89 + 0.03	b	1.18 + 0.04	d	0.74 + 0.01	b
	160	0.73 + 0.03	c	0.90 + 0.00	b	0.74 + 0.02	bc	0.67 + 0.02	b
	240	0.58 + 0.04	b	0.75 + 0.01	a	0.66 + 0.00	b	0.37 + 0.01	a
	320	0.29 + 0.02	a	0.80 + 0.02	a	0.78 + 0.01	c	0.72 + 0.03	b
D-ara	0	1.84 + 0.05	a	1.84 + 0.05	a	0.55 + 0.07	a	0.55 + 0.07	a
	80	2.14 + 0.05	a	1.79 + 0.07	a	1.91 + 0.16	b	1.03 + 0.00	b
	160	3.29 + 0.05	b	1.66 + 0.09	a	3.01 + 0.10	c	1.13 + 0.03	b
	240	3.09 + 0.23	b	1.60 + 0.24	a	3.17 + 0.00	c	0.69 + 0.02	a
	320	1.64 + 0.01	a	2.13 + 0.05	a	3.39 + 0.07	c	1.23 + 0.02	b
C-glu	0	0.20 + 0.01	c	0.20 + 0.01	c	0.12 + 0.00	c	0.12 + 0.00	b
	80	0.17 + 0.00	bc	0.11 + 0.01	b	0.17 + 0.01	d	0.08 + 0.00	a
	160	0.13 + 0.01	b	0.10 + 0.01	b	0.04 + 0.00	a	0.06 + 0.01	a
	240	0.03 + 0.00	a	0.07 + 0.01	a	0.07 + 0.00	b	0.12 + 0.01	b
	320	0.13 + 0.02	b	0.10 + 0.00	b	0.04 + 0.00	a	0.08 + 0.01	a
Pt-gal	0	3.20 + 0.06	b	3.20 + 0.06	b	1.15 + 0.07	a	1.15 + 0.07	a
	80	3.36 + 0.08	b	3.12 + 0.07	b	3.63 + 0.06	b	2.05 + 0.07	b
	160	3.70 + 0.06	b	2.70 + 0.08	a	3.37 + 0.07	b	2.34 + 0.07	b
	240	3.46 + 0.17	b	2.32 + 0.08	a	3.67 + 0.06	b	1.15 + 0.01	a
	320	1.32 + 0.10	a	3.14 + 0.08	b	4.12 + 0.51	b	2.23 + 0.15	b
C-ara	0	0.35 + 0.01	c	0.35 + 0.01	b	0.18 + 0.01	a	0.18 + 0.01	a
	80	0.39 + 0.01	c	0.31 + 0.01	ab	0.41 + 0.01	d	0.25 + 0.01	b
	160	0.30 + 0.02	b	0.33 + 0.01	ab	0.23 + 0.01	b	0.21 + 0.01	ab
	240	0.27 + 0.01	b	0.28 + 0.03	a	0.26 + 0.00	bc	0.18 + 0.00	a
	320	0.14 + 0.00	a	0.31 + 0.01	ab	0.30 + 0.01	c	0.26 + 0.02	b
Pt-glu	0	0.41 + 0.01	b	0.41 + 0.01	c	0.52 + 0.01	d	0.52 + 0.01	b
	80	0.35 + 0.02	b	0.19 + 0.01	ab	0.29 + 0.01	c	0.21 + 0.02	a
	160	0.31 + 0.01	b	0.17 + 0.02	a	0.29 + 0.00	c	0.07 + 0.00	a
	240	0.10 + 0.02	a	0.20 + 0.02	ab	0.23 + 0.02	b	0.47 + 0.08	b
	320	0.63 + 0.04	c	0.24 + 0.01	b	0.11 + 0.01	a	0.25 + 0.01	a
Pn-gal	0	0.37 + 0.01	c	0.37 + 0.01	c	0.17 + 0.01	a	0.17 + 0.01	a
	80	0.42 + 0.01	c	0.36 + 0.03	bc	0.41 + 0.01	b	0.29 + 0.01	c
	160	0.27 + 0.01	b	0.31 + 0.01	abc	0.30 + 0.01	ab	0.31 + 0.01	c
	240	0.28 + 0.02	b	0.28 + 0.02	a	0.25 + 0.01	ab	0.17 + 0.01	a
	320	0.12 + 0.02	a	0.29 + 0.01	ab	0.34 + 0.07	ab	0.25 + 0.01	b
Pt-ara	0	1.07 + 0.02	b	1.07 + 0.02	a	0.51 + 0.02	a	0.51 + 0.02	a
	80	1.36 + 0.02	bc	1.11 + 0.07	a	1.36 + 0.02	b	0.70 + 0.02	b
	160	1.51 + 0.02	c	0.89 + 0.06	a	1.96 + 0.07	c	0.78 + 0.02	c
	240	1.38 + 0.12	c	0.87 + 0.06	a	1.49 + 0.05	b	0.54 + 0.01	a
	320	0.63 + 0.01	a	1.13 + 0.05	a	1.43 + 0.01	b	0.75 + 0.00	bc
Pn-glu	0	0.18 + 0.01	c	0.18 + 0.01	b	0.11 + 0.02	ab	0.11 + 0.02	a
	80	0.19 + 0.01	c	0.11 + 0.02	a	0.18 + 0.01	b	0.10 + 0.01	a
	160	0.10 + 0.00	b	0.11 + 0.01	a	0.08 + 0.00	a	0.06 + 0.00	a
	240	0.03 + 0.01	a	0.07 + 0.01	a	0.06 + 0.01	a	0.08 + 0.03	a
	320	0.09 + 0.02	b	0.13 + 0.01	ab	0.07 + 0.02	a	0.09 + 0.02	a
M-gal	0	8.05 + 0.16	cd	8.05 + 0.16	c	4.52 + 0.04	a	4.52 + 0.04	ab
	80	8.69 + 0.22	d	8.00 + 0.23	c	9.05 + 0.14	d	6.17 + 0.31	cd
	160	6.95 + 0.11	bc	6.53 + 0.20	b	6.44 + 0.14	c	7.02 + 0.17	d
	240	5.84 + 0.36	b	5.49 + 0.15	a	6.39 + 0.17	bc	4.06 + 0.10	a
	320	2.74 + 0.23	a	6.60 + 0.16	b	5.59 + 0.19	b	5.48 + 0.24	bc
Pn-ara	0	0.10 + 0.00	c	0.10 + 0.00	a	0.06 + 0.01	a	0.06 + 0.01	a
	80	0.12 + 0.01	d	0.11 + 0.03	a	0.13 + 0.01	b	0.08 + 0.00	b
	160	0.11 + 0.00	cd	0.11 + 0.01	a	0.12 + 0.02	b	0.14 + 0.01	c
	240	0.08 + 0.00	b	0.11 + 0.01	ab	0.10 + 0.01	ab	0.08 + 0.00	b
	320	0.05 + 0.00	a	0.08 + 0.01	a	0.09 + 0.01	ab	0.07 + 0.00	ab
M-glu	0	0.87 + 0.01	c	0.87 + 0.01	d	1.81 + 0.08	d	1.81 + 0.08	d
	80	0.88 + 0.02	c	0.45 + 0.01	a	0.87 + 0.01	c	0.75 + 0.02	b
	160	0.59 + 0.01	b	0.50 + 0.02	b	0.41 + 0.01	b	0.28 + 0.01	a
	240	0.15 + 0.02	a	0.55 + 0.01	c	0.42 + 0.03	b	1.33 + 0.06	c
	320	1.09 + 0.06	d	0.50 + 0.01	b	0.19 + 0.03	a	0.70 + 0.01	b
M-ara	0	3.11 + 0.05	b	3.11 + 0.05	a	2.51 + 0.12	a	2.51 + 0.12	a
	80	3.99 + 0.18	c	3.20 + 0.38	a	3.90 + 0.08	b	2.69 + 0.15	a
	160	3.05 + 0.08	b	2.72 + 0.18	a	3.12 + 0.08	a	2.97 + 0.10	a
	240	2.59 + 0.25	b	2.49 + 0.11	a	2.97 + 0.16	a	2.28 + 0.09	a
	320	1.61 + 0.03	a	2.48 + 0.06	a	2.55 + 0.21	a	2.50 + 0.21	a
Ant_tot	0	25.15 + 0.43	bc	25.15 + 0.43	c	13.83 + 0.45	a	13.83 + 0.45	a
	80	27.29 + 0.72	bc	23.92 + 1.00	bc	28.17 + 0.35	b	17.34 + 0.38	b
	160	28.10 + 0.21	c	20.67 + 0.55	ab	26.27 + 0.19	b	18.61 + 0.50	b
	240	24.19 + 1.23	b	18.38 + 0.88	a	26.60 + 0.60	b	12.82 + 0.31	a
	320	13.83 + 0.26	a	22.75 + 0.44	bc	26.04 + 1.12	b	17.37 + 0.77	b

Values are means ± standard error, n=6 Means follower by different letters are significantly different at P<0.05% (Tukey's test). continue 1

(a) small letters in the same column refer to the influence of blanching time within the same product

Table 5.10: continue 1

	BL_time (s)	H_time (s) 5 BL+H		H_time (s) 5 H+BL		H_time (s) 15 BL+H		H_time (s) 15 H+BL	
GAL	0	16.68 + 0.28	b	16.68 + 0.28	c	9.52 + 0.17	a	9.52 + 0.17	a
	80	17.48 + 0.43	b	16.39 + 0.41	c	18.77 + 0.38	b	11.3.25 + 0.29	b
	160	18.29 + 0.18	b	13.98 + 0.35	ab	16.75 + 0.09	b	12.87 + 0.33	b
	240	16.29 + 0.62	b	12.02 + 0.45	a	17.54 + 0.37	b	7.83 + 0.09	a
	320	10.48 + 0.33	a	15.42 + 0.38	bc	17.81 + 0.84	b	11.26 + 0.50	b
ARA	0	6.46 + 0.12	b	6.46 + 0.12	a	3.82 + 0.23	a	3.82 + 0.23	ab
	80	8.01 + 0.26	bc	6.51 + 0.56	a	7.70 + 0.06	b	4.74 + 0.14	abc
	160	8.25 + 0.01	c	5.70 + 0.22	a	8.42 + 0.27	b	5.23 + 0.16	c
	240	7.41 + 0.60	bc	5.33 + 0.42	a	7.97 + 0.20	b	3.76 + 0.12	a
	320	4.07 + 0.03	a	6.12 + 0.05	a	7.75 + 0.29	b	4.81 + 0.25	bc
GLU	0	2.01 + 0.03	d	2.01 + 0.03	c	2.80 + 0.10	d	2.80 + 0.10	d
	80	1.80 + 0.04	c	1.03 + 0.04	a	1.70 + 0.03	c	1.28 + 0.05	b
	160	1.56 + 0.02	b	1.00 + 0.01	a	1.11 + 0.02	b	0.52 + 0.02	a
	240	0.50 + 0.02	a	1.03 + 0.01	a	1.10 + 0.04	b	2.22 + 0.10	c
	320	2.49 + 0.06	e	1.22 + 0.02	b	0.54 + 0.05	a	1.30 + 0.02	b
MALV	0	12.02 + 0.21	cd	12.02 + 0.21	c	8.83 + 0.23	a	8.83 + 0.23	ab
	80	13.55 + 0.41	d	11.64 + 0.61	bc	13.81 + 0.22	b	9.61 + 0.44	b
	160	10.59 + 0.04	bc	9.74 + 0.40	ab	9.96 + 0.05	a	10.27 + 0.28	b
	240	8.57 + 0.62	b	8.52 + 0.25	a	9.77 + 0.35	a	7.66 + 0.24	a
	320	5.43 + 0.31	a	9.57 + 0.10	a	8.34 + 0.44	a	8.68 + 0.46	ab
DELPH	0	6.32 + 0.10	a	6.32 + 0.10	bc	4.14 + 0.22	a	4.14 + 0.22	c
	80	6.43 + 0.15	a	6.00 + 0.15	abc	6.62 + 0.01	b	3.25 + 0.08	ab
	160	10.37 + 0.15	c	5.34 + 0.17	ab	9.20 + 0.20	c	3.71 + 0.09	bc
	240	9.43 + 0.36	bc	4.94 + 0.45	a	10.08 + 0.18	cd	3.01 + 0.01	a
	320	8.23 + 0.47	b	6.98 + 0.17	c	10.51 + 0.13	d	4.01 + 0.09	c
PETUN	0	4.67 + 0.09	b	4.67 + 0.09	b	2.17 + 0.09	a	2.17 + 0.09	a
	80	5.07 + 0.12	bc	4.41 + 0.14	b	5.27 + 0.06	b	2.95 + 0.05	b
	160	5.52 + 0.03	c	3.75 + 0.01	a	5.62 + 0.00	b	3.19 + 0.09	b
	240	4.93 + 0.27	bc	3.39 + 0.16	a	5.38 + 0.09	b	2.16 + 0.10	a
	320	2.57 + 0.13	a	4.51 + 0.14	b	5.65 + 0.53	b	3.22 + 0.16	b
CYAN	0	1.51 + 0.03	d	1.51 + 0.03	c	0.65 + 0.02	a	0.65 + 0.02	a
	80	1.52 + 0.03	d	1.30 + 0.03	b	1.75 + 0.05	c	1.07 + 0.01	b
	160	1.15 + 0.05	c	1.32 + 0.01	b	1.01 + 0.03	b	0.94 + 0.03	b
	240	0.87 + 0.04	b	1.09 + 0.02	a	0.99 + 0.01	b	0.67 + 0.02	a
	320	0.55 + 0.01	a	1.20 + 0.02	ab	1.12 + 0.01	b	1.06 + 0.04	b
PEON	0	0.64 + 0.01	c	0.64 + 0.01	a	0.34 + 0.02	a	0.34 + 0.02	a
	80	0.72 + 0.01	c	0.57 + 0.07	a	0.71 + 0.01	b	0.48 + 0.02	b
	160	0.48 + 0.01	b	0.53 + 0.03	a	0.49 + 0.03	ab	0.50 + 0.01	b
	240	0.40 + 0.02	b	0.46 + 0.02	a	0.40 + 0.01	a	0.33 + 0.03	a
	320	0.26 + 0.04	a	0.49 + 0.01	a	0.50 + 0.10	ab	0.41 + 0.02	ab
CA	0	2.60 + 0.06	a	2.60 + 0.06	b	3.70 + 0.02	b	3.70 + 0.02	d
	80	1.66 + 0.04	a	1.83 + 0.03	a	1.89 + 0.13	a	2.37 + 0.05	b
	160	6.99 + 0.11	b	1.71 + 0.07	a	5.78 + 0.09	d	1.53 + 0.04	a
	240	6.10 + 0.36	b	3.00 + 0.05	c	7.29 + 0.01	e	4.06 + 0.10	d
	320	11.79 + 0.03	c	2.75 + 0.07	bc	4.77 + 0.01	c	3.13 + 0.12	c

Values are means ± standard error, n=6
Means follower by different letters are significantly different at P<0.05% (Tukey's test).
(a) small letters in the same column refer to the influence of blanching time within the same product

ID: D-gal=Delphynidin-3-O-galactoside; D-glu=Delphinidin-3-O-glucoside; C-gal=Cyanidin-3-O-galactoside; D-ara=Delphynidin-3-O-arabinoside; C-glu=Cyanidin-3-O-glucoside; Pt-gal=Petunidin-3-O-galactoside; Pt-glu=Petunidin-3-O-glucoside; C-ara=Cyanidin-3-O-arabinoside; Pn-galPeonidin-3-O-galactoside; Pt-araPetunidin-3-O-arabinoside; M-gal=Malvidin-3-O-galactoside; Pn-glu=Peonidin-3-O-glucoside; M-glu=Malvidin-3-O-glucoside; Pn-ara=Peonidin-3-O-arabinoside; M-ara=Malvidin-3-O-arabinoside; GAL= Total galactosides, ARA=Total arabinosides, GLU=Total glucosides, MALV=Total malvidins, DELPH=Total delphynidins, PETUN= Total petunidins, CYAN=Total cyanidins, PEON=Total peonidins, CA=Chlorogenic acid

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6. TOPIC 4

Study of the microstructure of blueberry fruits and tissue−components from derived homogenate products

Histochemical features of raw and thermally treated blueberry fruits and homogenate products were analyzed by brightfield, darkfield, phase contrast and fluorescence microscopy modes. To enable the sampling of homogenate particles, sedimentation trials were carried out in sucrose and aqueous separating media and qualitative textural classes of particles with similar density and morphometric features were obtained. The properties of both intrinsic and extrinsic fluorophores were exploited to reveal microstructure details by fluorescence microscopy mode.

6.1. Material and methods

Microstructure study was carried out on samples from Trial 3

6.1.1. Epi-Fluorescence Microscope System

Samples were examined with a BX43 upright microscope (Olympus, Japan) equipped with a turret condenser for brightfield (BF), darkfield (DF), and phase contrast (Ph) observation and with a reflected fluorescence (F) system housing a mercury burner and three mirror units F1, F2 and F3 (Table 6.1). Pictures were acquired with a CCD color camera XC30 (Olympus, Japan). Images were processed with a Digital Imaging Software cellSens Dimension Version 1.7 (Olympus, Japan).

Table 6.1: Filter configuration of fluorescence mirror units

	Mirror Unit	Excitation filter	Emission filter	Dichromatic mirror
F1	U-FUN	360-370 nm (Ultraviolet)	420 nm (Blue)	410 nm
F2	U-FBW	460-495 nm (Blue)	510 nm (Green)	505 nm
F3	U-FGW	530-555 nm (Green)	575 nm (Red)	570 nm

6.1.2. Fruit tissue sampling

Tissue samples were excised from different regions of blueberry fruits (Figure 6.1).

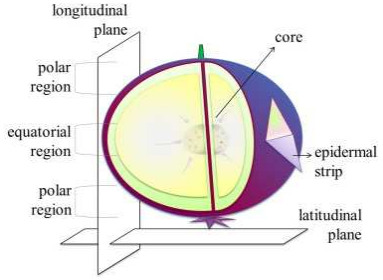

Figure 6.1: Planes of section of blueberry fruit

Epidermal strips were taken from polar and equatorial regions of frozen berries with the aid of a finely pointed titanium tweezer (Sigma). Longitudinal and latitudinal thick sections (3-5 mm) were cut at different depths from the surface (tangential sections) to the core (mid-cross sections) of frozen berries by means of a sharp razor blade. Transverse thin sections (50-100 µm) of tissue portions were generated using a Vibroslice HA752 Vibrating Microtome equipped with a Tissue Bath Cooler 7600 (Campden Instruments Ltd., Leicestershire, UK). At least 10 fruits were analyzed for each raw/thermally treated sample.

6.1.3. Homogenate sampling

Frozen stored homogenates (2 × 14 ml aliquots) were quickly thawed in a water bath at 22 °C and the microstructure was analyzed of whole homogenates (H) and homogenate fractions (HF) obtained by density gradient separation in sucrose solutions (HF_DG) or by decantation in

aqueous solutions (HF_D). Both H and HF samples were studied in duplicate and three drops (one drop, approx 50 µl) for each replica were individually examined by microscopy.

6.1.3.1. Density gradient separation in sucrose solutions

Separation of particles in sucrose step density gradients is a method typically applied to fractionate subcellular components from plant materials (Fritsch, 1975). In this procedure, concentrated stock solutions of sucrose are prepared and further diluted to provide gradient steps of a suitable lower density, compatible with density of the particles to be separated: particles move through this gradient and stop forming a layer at the point at which their density matches that of the surrounding medium.
Single sucrose concentrations were preliminary tested to assess the sugar density range compatible with the densities of homogenate particles and separation trials were carried out in discontinuous sucrose gradients produced by overlayering and underlayering techniques.
For the assessment of density range, a 60 % (w/v) working solution (WS) of sucrose (Merck) was prepared and 30-20-15-10-7.5-3.75-1 % (w/v) gradient solutions were prepared from the WS by diluting with water. For each sucrose concentration, 10 g of syrup were weighted in 30-ml centrifuge tubes and 10 ml of water-diluted (1:1 w/w) blueberry homogenate were layered on the top. Samples were centrifuged at 3,000 × g for 10 min or 10,000 × g for 20 min in a thermostated centrifuge (Heraeus Biofuge Stratos, Kendro, Germany) at 20°C.
Overlayering technique: starting from densest sucrose solution (60% w/v), 15-ml aliquots of solutions of successive lower densities (30%, 15%, 5%, 1% w/v) were gently layered on the top of 100-ml glass graduated cylinders, using a glass pipette, just above the meniscus of the liquid and against the wall of the cylinder to minimise any mixing. Once a steady state was established, 10 g of water-diluted (1:1 w/w) blueberry homogenate were layered on the top.
Underlayering technique: for each sample, 10 g of water-diluted (1:1 w/w) homogenate were weighted into the barrel of a 50-ml Plastic Syringe (Terumo Europe, Leuven; Belgium), the plunger was added and 10-ml aliquots of successively denser sucrose solutions (1%, 5%, 15%, 30% and 60% w/v) were drawed and underlayered.
Particles from blueberry homogenates were allowed to settle by gravity through the sucrose gradients at room temperature for 60 minutes.

6.1.3.2. Decantation in aqueous solutions

Decantation trials were performed according to the protocol reported in section 4.1.1.5., with few modifications. One gram of homogenate product for each sample was weighed in 25-ml glass cylinders, and 20 ml of distilled water were added. Diluted samples were mixed on a Vortex for 10 s and after 5 min they were mixed up-and-down ten times and allowed to settle for 48 h in a thermostatic chamber at 4 °C.

6.1.4. Staining procedure

Intrinsic fluorescence properties (autofluorescence) of fruit natural metabolites were exploited to reveal tissue components without the use of staining (Talamond et al., 2015; Roshchina, 2012) and two fluorescent brightening agents, Calcofluor White and Naturstoff reagent A, were introduced to enhance details detection (Table 6.2).
All the tissue samples were collected and immediately placed on ice in a phosphate-buffered saline system (PBS, pH 6.8) to preserve cellular osmolarity and stabilize fluorescence lifetime.

Fruit tissue samples were immersed in the staining solution for 10 min, rinsed in PBS and observed under microscope. Homogenate particles were diluted 1:1 on the glass slide with the staining solution and allowed to stand for one minute with the stain before the coverslip was placed over the specimen and the excess fluid was removed with a paper towel.

6.1.4.1. Phosphate-buffered saline system (PBS; pH 6.8; 0.15M NaCl)

The phosphate-buffered saline system **(**PBS) at pH 6.8 was prepared by mixing in a 500-ml volumetric flask 10.45 ml of 1 M KH_2PO_4 (Sigma-Aldrich, P8706), 14.55 ml of 1 M K_2HPO_4 (Sigma-Aldrich, P8584), 15 ml of 5 M NaCl (Sigma-Aldrich, S6546) and bringing to volume with water, according to the PBS1 data sheet instruction of reagents supplier (Sigma-Aldrich)

6.1.4.2. Calcofluor White M2R (CFW)

CFW (Fluorescent Brightener 28) is a fluorescent dye which binds to β-linked glucosidic polymers such as chitin and cellulose and is usually applied to selectively detect cell walls of fungi, bacteria, algae and higher plants. A stock solution 1% (w/v) CFW (Sigma-Aldrich) was prepared by stirring 100 mg of the stain in 10 ml distilled water with few drops of 1 N NaOH. A 0.1 % CFW in PBS working solution was prepared from the stock at room temperature (Herth & Schnepf, 1980; Rothamsted, Bioimaging).

6.1.4.3. Naturstoff reagent A (NA)

NA (2−aminoethyl diphenylborinate) reagent is a fluorescence inducing agent largely applied in chromatography to visualize flavonoid compounds, also tested for histochemical detection of flavonoids in plant and animal tissues (Brunetti *et al*., 2013; Agati*et al*., 2012; Ernst *et al*, 2010; Agati *et al*., 2009). A 2.5 % (w/v) NA (Sigma-Aldrich) in ethanol stock solution was prepared and a 0.1% NA in PBS working solution was diluted from the stock (Hutzler *et al*., 1998). Stock solutions were stored at −20 °C in small aliquots wrapped with aluminum foils. Working solutions were freshly prepared from the stocks immediately before use.

Table 6.2: Fluorescence properties of natural compounds and brightening agents

	Excitation wavelength (nm)	**Emission wavelength (nm)**
Natural compound*		
Chlorophyll	Blue (430-450, up to 550)	Red (max 685)
Cutin	UV (365)	Blue/white
Guard cells	Violet (450)	Green (520)
Lignin	UV (365)	Blue (450–480); Green (510–520)
Fluorescence enhancer		
Calcofluor White M2R	UV; Blue	Blue; Green
Naturstoffe reagent	UV; Blue	Blue; Green

*Adapted from: Ruzin (2009)

6.2. Results and discussion

6.2.1. Microstructure of blueberry fruit

Blueberry fruits of "Brigitta" cultivar were medium to large in size, slightly flattened in shape with a longitudinal diameter of 12.3±0.3 mm, a latitudinal diameter of 17.5±0.2 mm and an average weight of 2.5±0.1g.

Microstructural elements of the berry fruits were detected by reflected light fluorescence microscopy by exploiting primary fluorescence properties of cutin, lignin and chlorophyll molecules. Besides, anthocyanin-pigmented cells were analyzed by transmitted light microscopy on fruit epidermal strips and thin sections (Figure 6.2).

A cuticular waxy layer was detected on the surface of the fruits. In the pedicellar area, stomata surrounded by peculiar waxy patterns were evidenced, as well as sporadic lenticel-like structured (Figure 6.2.A1).

"Brigitta" epidermis consisted on one external layer of thick-walled, flattened cells, closely associate and small in size (10-15 μm thick and 40-50 μm large, approx.) plus two/three interior layers of cells, more loosely associated and bigger in size (15-20 μm thick and 50-70 μm large, approx.). Small variations can be found among blueberry cultivars with respect to the number of pigmented layers of cells in the epidermis (Fava et al, 2006; Sapers & Phyllips, 1985). In "Brigitta" fruit, anthocyanin pigments were mainly localized in the vacuoles of cells belonging to the outer layer (epidermis) with few pigmented vacuolar vesicles associated to the hypodermis cells (Figure 6.2.A2). Fluorescence panoramic views of the fruit peel revealed that epidermis exhibited a specific cellular pattern, with cell packets of 4-6 elements enclosed within a perimeter of thicker walls (Figure 6.2.A1). Cell packets are considered descended from a single cell, hence describing the history of divisions undergone during development (Raczynska-Szajgin & Nakielski, 2014).

Underneath the epidermis, cells steeply increase in size (200 μm diameter, approx.) and changed from epidermal to parenchymal type (isodiametric, thin walled, loosely associated cells), hence creating a discontinuity/fault between layers. Figure 6.3.a reports a cross-section of blueberry fruit where the architecture of tissue cells, increasing in size from the periphery to the core of the fruit, is displayed. In the sub-epidermal layer, isodiametric stone cells (100 μm diameter, approx.) and vascular bundles were positioned, providing support to the soft tissues. Vascular bundles (10-12 μm diameter, approx.) stood out among the mesocarp cells due to the intense lignin fluorescence of their spiral and annular secondary thickenings. Seeds (1-2 mm, approx.) were detected in the ovary region, encased within a layer of elongated stone cells (Figure 6.2.B. 1, 2, 3, 4). A similar pattern of stone cells, isodiametric and isolated in the subepidermal layer or elongated and in clusters in the endocarp area was reported by Allan-Wojtas et al. (2001) and related to the firmness of the fruits.

6.2.2. Microstructure of blueberry purée products

The overall microstructure of blueberry purées was analyzed by fluorescence microscopy, enhancing the fluorescence of cell wall structures using a Calcofluordye, specific for cellulose components (Figure 6.3.b, 6.4 and 6.5)

Simple blending of blueberry tissues produced large tissue fragments ($0.5<x<2$ mm), entangled with each other due to sticky/rough surfaces (broken hedges). Within these fragments epidermal

portions (>1mm) were included, with well-preserved pigmented vacuoles. Longer H-time produced smaller tissue fragments without changing the overall particle texture (Figure 6.3.b).

What is noticeable, larger tissue fragments were always associated to sclereids and vascular components of the fruits, as well as epidermal fragments. We can infer that these elements acted as supporting structures protecting surrounding cells from external forces during processing as well as in nature.

At the lowest BL-time (BL=80s), no significant differences were detected between the overall particle microstructures of unblanched and thermally treated products, whatever the blanching/blending operation order applied (Figure 6.4 and 6.5, BL=80s).

On the contrary, for BL-time≥160s, purées originated from non-pretreated (HBL) or preliminary blanched (BLH) blueberries exhibited a completely different pattern of particles.

For BL=160s, blanched-blended purées (BLH, Figure 6.4) mainly consisted of small, smooth clusters of few cells (500 μm diameter, approx.), associated to several individual cells, characterized for the most part by intact hedges. Small fragments (≤ 500 μm) of epidermis with empty vacuoles were detected. With the increase of BL-time (BL=240s), the particle phase of BLH purées consisted almost entirely of individual cells, rounded or elongated in shape. Continuity of cell walls was preserved, but fluorescent filaments started to unravel from their surface. With the longest thermal treatment (BL=320s) the pattern of particles did not change but cell walls appeared lightened, almost liquefied, in few cases ripped off with soft frayed hedges. Increasing amounts of fluorescent fibrillary materials were detected associated to cell walls and dispersed in the serum phase.

In Figure 6.6, details of fluorescent fibrils unraveled from the cell wall of extensively blanched BLH purées are visible (a), as well as fibrils already dissolved in the serum phase (c). Furthermore, by combining transmitted and reflected light sources, details inside the cells were revealed indicating that, even in these conditions, cytoplasmic materials were still enveloped within walls (b).

Longer H-time (H5 *vs* H15) produced more pronounced deformations on cells but did not significantly change the overall purée texture.

In contrast, for BL=160s blended-blanched purèes (HBL, Figure 6.5) mainly consisted in large clusters of cells ($0.5<x<2$ mm, approx.) very similar in size and shape to unblanched purée particles. With the increase of BL-time, cell cluster decreased in size but, compared to preliminary blanched purées, in these products much more broken edges and ripped off wall fragments were detected as well as cytoplasmic components attached to the surface of cells.

In figure 6.6, details of broken cells in extensively blanched HBL purées (BL=320s) are reported (a), as well as cell debris dissolved in the serum phase (c). Cells were empty, and cytoplasmic material leaked out (b). Also in this case longer H-time produced smaller tissue fragments without changing the overall particle texture.

Hence, if we analyze the different microstructures of purée particles, we can deduce that mechanical treatments produced the failure of tissues between cells in preliminary blanched fruits, and through cells in unblanched tissues, hence resulting in smaller smooth particles in BLH purées and larger rough particles in HBL products. Heat-induced softening of pectins in the middle lamella of BL fruits can explain the different behavior of BL tissues during subsequent purée processing.

These data are in agreement with previous observations on the microstructure of tomato, carrots and broccoli food dispersions obtained starting from the same vegetable material but applying

different unit operations (Lopez-Sanchez et al., 2011a, b; Lopez-Sanchez et al., 2015; Moelants et al., 2014a; Christiaens et al., 2012). Furthermore, loosening of cellulose fibrils in tangential epidermal cell walls of steam blanched blueberries had been previously reported by Fava et al. (2006). Nevertheless, detachment of cellulosic fibrils from cell walls of plant-food suspensions had not been described before.

To conclude, by introducing a preliminary blanching step in the production of blueberry purées (BLH products), single cells were obtained, the continuity of membranes and walls was better preserved but the ultrastructure of cell walls was deeply altered with cellulose fibrils detached from the wall surface. Hence BLH particle phase was characterized by high phase volume, low amount of free cytoplasmic material and, what is noticeable, by a widespread network of fibrillary cellulose increasing the active surface area of the matrix.

Besides, in blended-homogenate products, larger and entangled cluster of cells were obtained but the continuity of both cell wall and membranes was lost and cytoplasmic material leaked out from the broken edges. Hence HBL particle phase was characterized by lower phase volume and higher amounts of free cytoplasmic materials.

If we consider that cellulose fibrils are characterized by a strong affinity for water (Fall et al., 2011), these data can contribute to explain the different hydration properties exhibited by HBL *vs* BLH blueberry purées (Topic 2).

Hence a link could be established between processing variables (operation order > BL-time > H-time), microstructural features of cell wall cellulosic materials and functional properties of derived blueberry homogenate matrices.

6.3. Conclusions

In BL+H products single cells were obtained, the continuity of membranes and walls was better preserved, and the ultrastructure of cell walls was deeply altered, with cellulose fibrils detached from wall surface producing a widespread network of fibrillary cellulose increasing the active surface area of the matrix. In H+BL products larger and entangled cluster of cells were obtained, the continuity of both cell wall and membranes was lost, cytoplasmic material leaked out from the broken edges, producing a lower phase volume of the particle phase as well as higher amounts of free cytoplasmic materials.

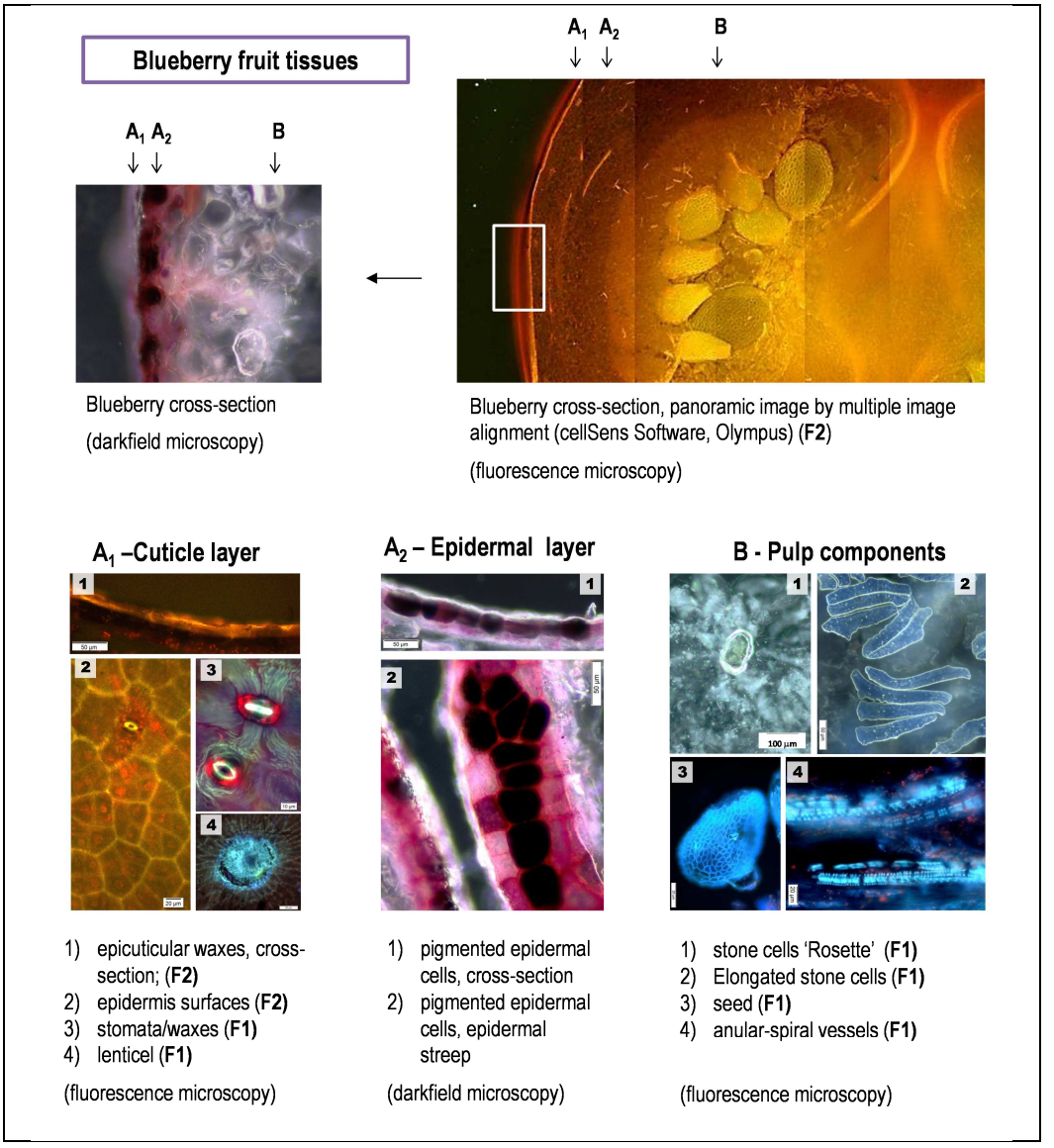

Figure 6.2: Microscopy images of tissue components characterizing blueberry fruits of "Brigitta"cultivar. Primary fluorescence. **F1**: Excitation = 360-370 nm, Emission = 420 nm; **F2**: Excitation = 460-495 nm, Emission = 510 nm

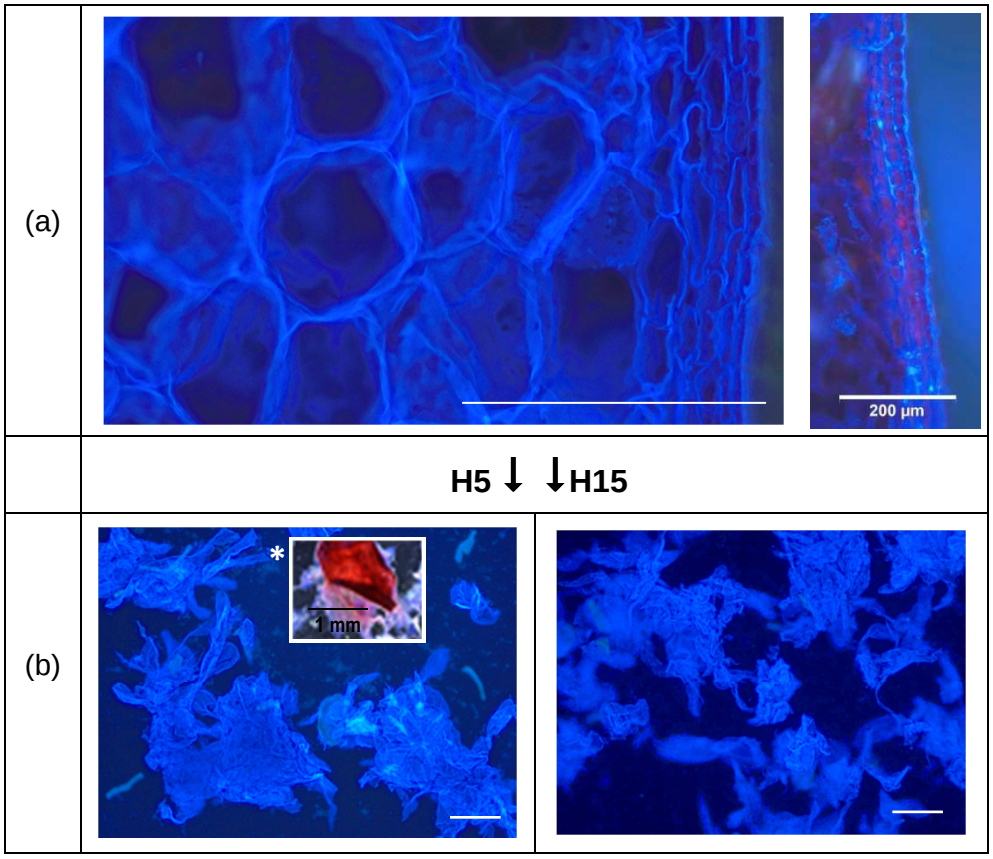

Figure 6.3: Fluorescence images showing the cell walls architecture in intact (a) and homogenized (b) blueberry fruits. Calcofluor White fluorescence enhancer (CFW) + Naturstoff reagent A (NA). **F1**: Excitation = 360-370 nm, Emission = 420 nm. Scale bar = 200 µm.
H=Homogenization
H-time= 5s and 15s
(*dark field micrograph: enlarged detail of a fragment of epidermis)

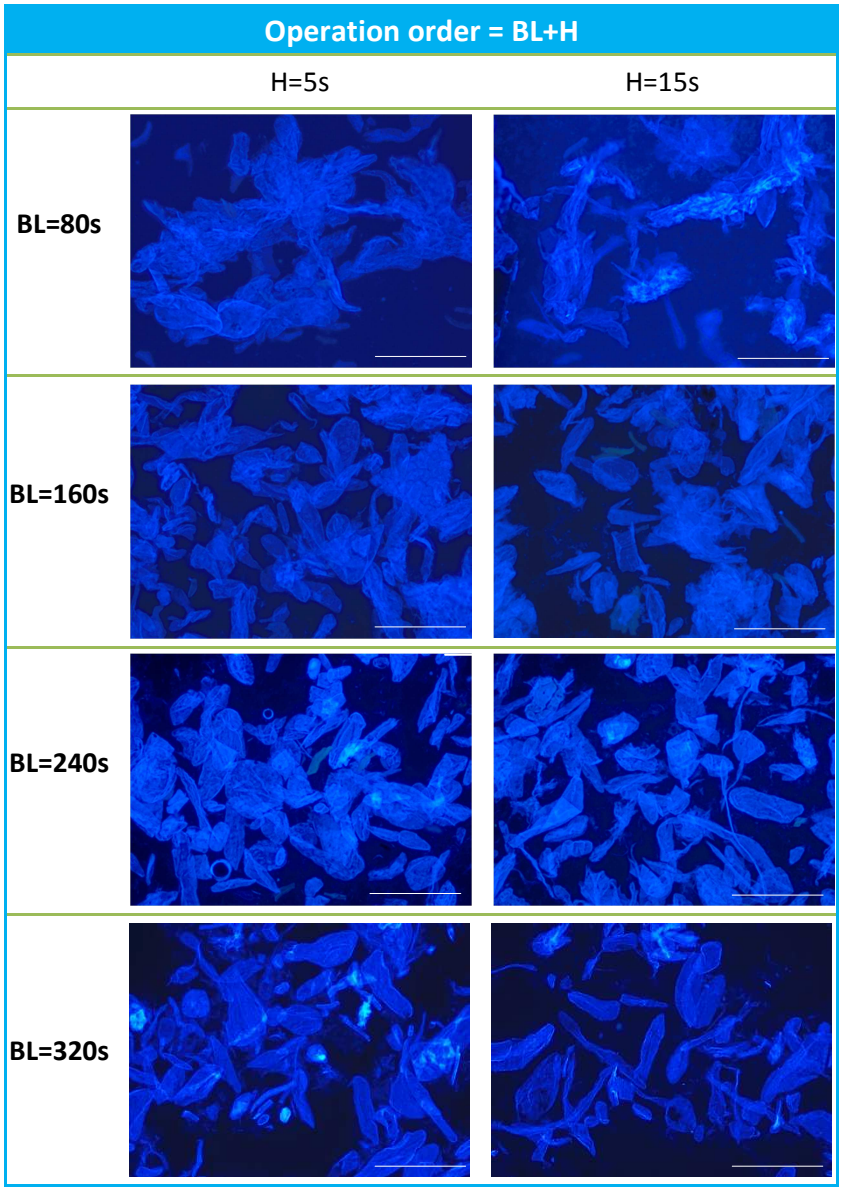

Figure 6.4:Fluorescence images of cell particles from thermally treated blueberry purées (operation order =BL+H) as a function of BL-time and H-time. Calcofluor White fluorescence enhancer (CFW)+ Naturstoff reagent A (NA). **F1**: Excitation = 360-370 nm, Emission = 420 nm.Scale bar = 500 µm
BL=Blanching; H=Homogenization

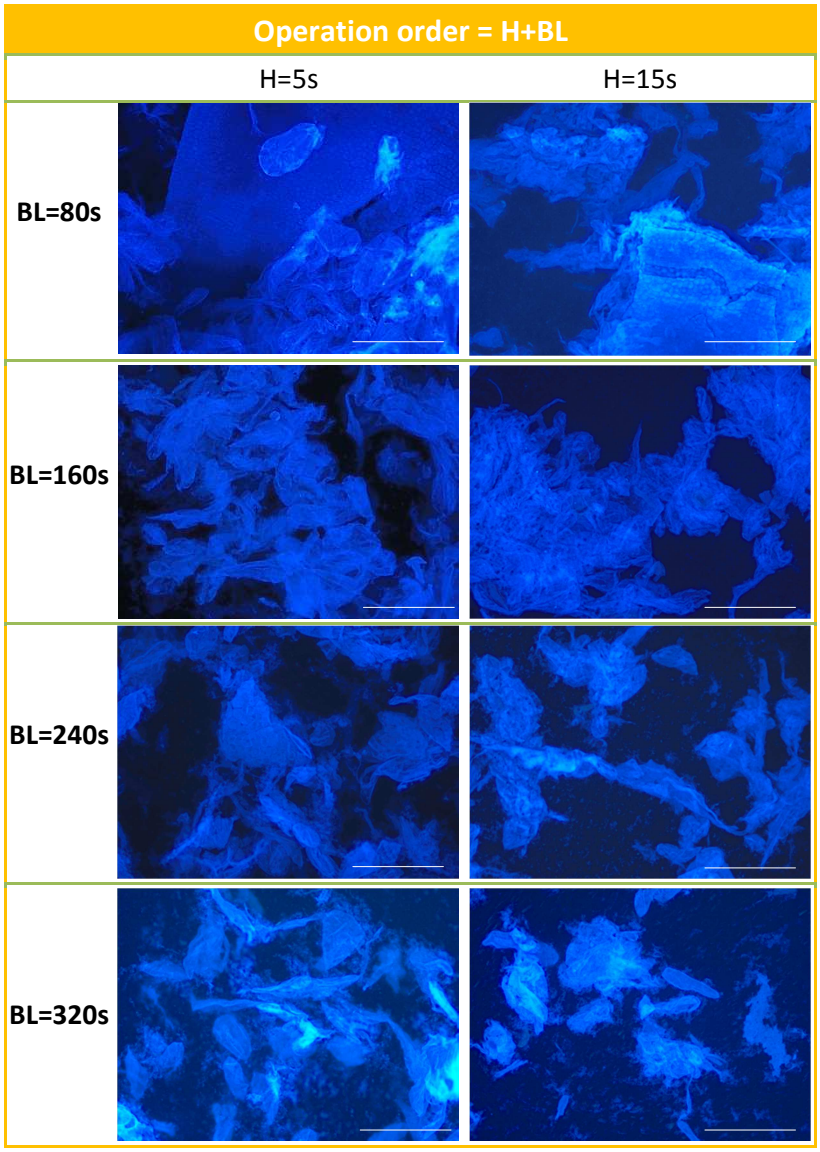

Figure 6.5: Fluorescence images of cell particles from thermally treated blueberry purées (operation order =H+BL) as a function of BL-time and H-time. Calcofluor White fluorescence enhancer (CFW) + Naturstoff reagent A (NA). **F1**: Excitation = 360-370 nm, Emission = 420 nm.Scale bar = 500µm
BL=Blanching; H=Homogenization.

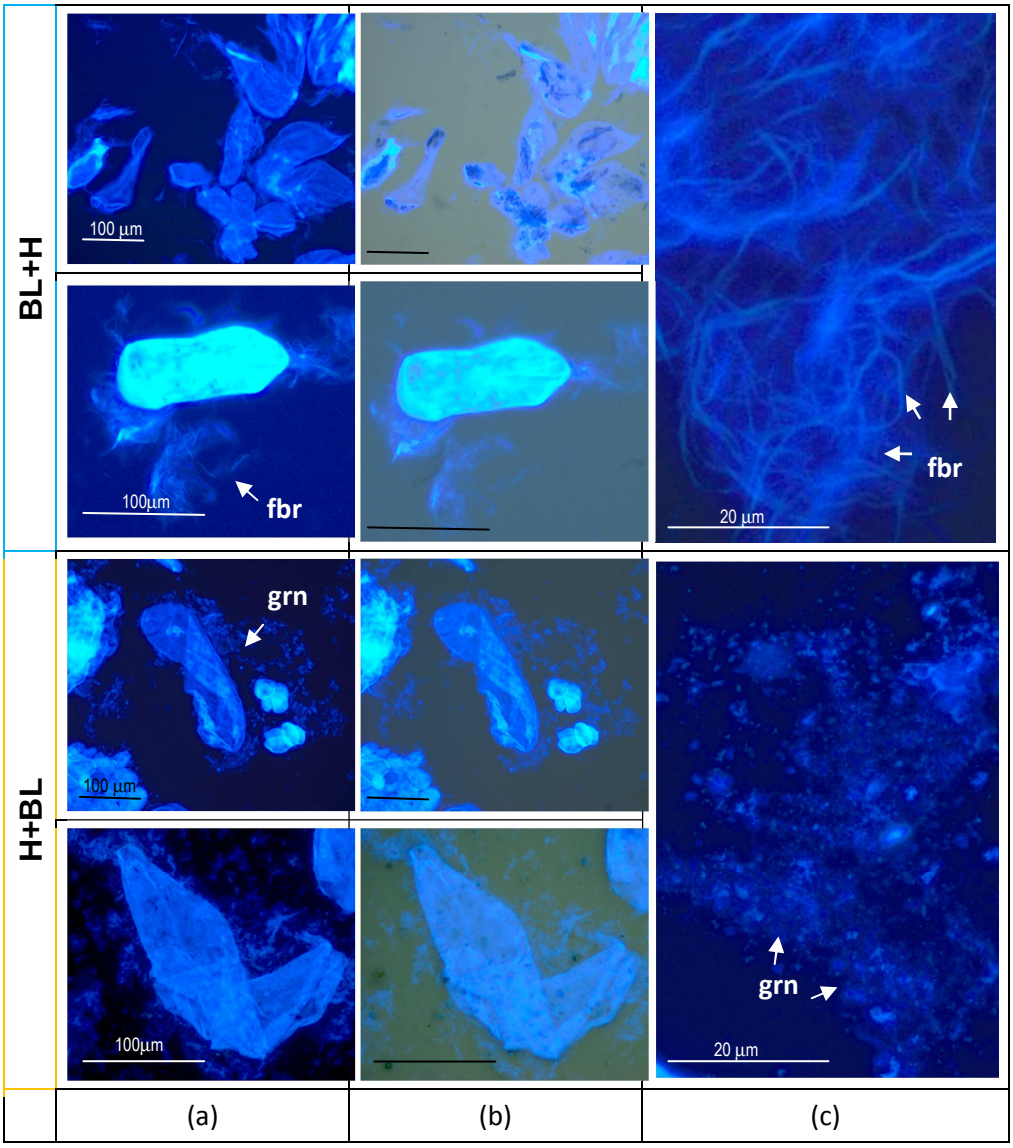

Figure 6.6: Fluorescence pictures of particles from blueberry purées (BL-time=320s) as a function of the operation order. (a) representative images under fluorescence (F) emission, (b) the same area of (a) but under combined bright field (BF) and F-emission, (c) enlarged details of cellulose fibrils (fbr) and cytoplasmic granules (grn) under F-emission. Calcofluor White fluorescence enhancer (CFW). **F1**: Excitation = 360-370 nm, Emission = 420 nm
BL=Blanching; H=Homogenization.

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